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STUDIES ON THE COCCACEAE

II. A STUDY OF THE GENERAL CHARACTERS OF THE MICROCOCCI

G. J. HUCKER

COMMONWEALTH MYCOLOGICAL INSTITUTE
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TECHNICAL BULLETIN No. 100

STUDIES ON THE COCCACEAE

II. A STUDY OF THE GENERAL CHARACTERS OF THE MICROCOCCI

G. J. HUCKER

SUMMARY

A correlation study of the general characteristics of 316 representative strains of the Micrococcus-Staphylococcus group indicates that there are no apparent natural division lines by which this group can be separated. No correlation could be found between two or more characters which would indicate that the types studied were so closely related that more than one genus should be formed.

A study of the constancy of various characters of this group shows that chromogenesis, nitrate reduction, ability to utilize ammonium salts as an only source of nitrogen, gelatin liquefaction, and action on milk are tests whereby the natural species of the group can be separated.

The fermentation of sugars, Gram reaction, habitat, hemolysis of blood, diastatic action, and oxygen requirements are characters which are less useful in the classification of the micrococci.

INTRODUCTION

In the study of the general characteristics of any group of bacterai the results are subject to two possible interpretations. The principal one is the notation of the biological and biochemical features with the view of giving additional information on the general activity and reactions of the entire group, and the other is to give more light on the taxonomic relationships of the group and the separation of the given group into natural subdivisions. In the main, any discussion of the latter must necessarily cover the former. Hence, the following discussion will deal with the general characters of the micrococci in the light of a possible natural classification of the organisms commonly included in this group.

In arranging the classification of groups of bacteria it is essential that such characters and diagnostic features be selected as separate the larger groups or genera in such a manner that the resulting subgroups will be composed of organisms of the same developmental origin and possessing similar and constant characters. In many of the

During the course of the investigation helpful and timely criticisms have been made by Dr. R. S. Breed of this Station and Dr. L. F. Rettger of Yale University.

outlines of classification of the Coccaceae mentioned in the previous paper of this series[1] the authors have merely served as collaborators, collecting the results secured by other investigators in an effort to arrange this heterogenous mass of material from the literature into a more logical form. In such instances, the collaborators often overlook the basic characteristics which are important in separating the particular groups studied. Bacteriological classification, like other taxonomic work, in order to give results of the greatest value must be based on a laboratory investigation of the relationships of the various species to each other and to the species of other closely related groups.

In the following sections of these investigations dealing with the Coccaceae the entire group will be considered as consisting of three sub-groups, *viz.*, (a) the *Streptococcus* group which is used in this place in a broad sense to include the streptococci, diplococci, gonococci, etc.; (b) the *Micrococcus*[2] group (staphylococci, rhodococci, and micrococci), including all of the irregular mass-forming cocci; and (c) the *Sarcina* group.

It is the object of this part of the investigation to make (a) a general survey of the irregular mass-forming group in an effort to ascertain its definite characteristics and to note if it can be divided into natural sub-divisions (genera) and, if so, which of our commonly used laboratory tests are applicable in making such a division; and (b) to select the more important characters by which the various species may be differentiated.

PREVIOUS STUDIES

Investigators have studied this group of cocci from various angles with varied and, in some instances, confusing results. The problem, in part, has been an endeavor to group the organisms in such a manner that species (or strains) of like habitat and other similar characteristics will be grouped together. Among the streptococci and micrococci both saprophytic and parasitic forms are found, and many of the investigators have regarded this difference of habitat as of primary importance. In many taxonomic studies of this group the object has been to find some general character which would separate the so-called free-living forms occurring under saprophytic conditions from the closely related species which have become permanently or tem-

[1]Technical Bulletin No. 99 of this Station.

[2]See later for a discussion regarding the usage of the terms *Micrococcus* and *Staphylococcus*. In the previous paper (*loc. cit.*) the names are used in the same sense as they are used by the authors quoted.

porarily adapted to a parasitic and, in some cases, pathogenic life. In such cases, it has usually been assumed that there are no "borderline cultures" which may, if the proper conditions are present, become parasites even if normally they are saprophytes, and *vice versa*. The investigators who are chiefly interested in public health bacteriology have many times failed to appreciate that organisms which are normally more or less infectious in nature may also occur as normal inhabitants of air, milk, soil, etc., and play some part in the normal changes which take place in these habitats. On the other hand, laboratory workers interested in industrial and agricultural bacteriology have usually considered only the organisms with which they have been thrown in contact and look upon the so-called "pathogenic types" as foreign to their field of bacteriology. It must not be forgotten that even tho the science of bacteriology is very naturally divided along the lines mentioned, it does not necessarily follow that the natural grouping of bacteria follows a similar course. It is evident that a study of the group of micrococci should include strains from all sources, and divisions should be sought which will divide the group, if it should be divided, into closely related and natural sub-groups.

In 1886, Longard[3] felt that certain differences must exist between the cocci from parasitic sources and those from milk. With this in mind he studied intensively a series of cultures from both sources. His results, however, indicated that the relationships between the two types were very close and, other than a few minor differences with regard to chromogenesis, the results were largely negative.

Moberg and Unna (1900) studied 145 strains of staphylococci, a large majority of which were from parasitic sources, and decided that the types under consideration should be classified into 23 different groups which could be characterized by size of cells and cell-masses, growth at various temperatures, chromogenesis, action upon milk, and liquefaction of gelatin. In this case the strains were all from one source and a large number of characters were considered of primary importance, the value of which in separating species may well be questioned.

In 1905, Gordon studied a large number of orange and white staphylococci and divided the group upon the basis of chromogenesis. He considered the white forms to be closely related to the streptococci, differing from them in respect to cell division, rate of gelatin liquefaction, nitrate reduction, and the fermentation of various sugars.

[3]See Bibliography, page 74.

In conclusion, he decided that nine tests could be adopted as of importance in grouping the cocci, the white forms in particular, *viz.*, liquefaction of gelatin, nitrate reduction, reduction of neutral red, action upon milk, and fermentation of lactose, maltose, glycerin, and mannite. The reduction of nitrates he considered a general characteristic of the white cocci.

Andrewes and Gordon (1905) adopted for the first time biometric methods for the study and classification of the cocci. The tests, which were principally the fermentation of the various carbohydrates, were all completed on a quantitative basis and frequency curves plotted for each test. The various peaks of these curves were taken as an indication of centers of species. This method of attack brought out many interesting results both in the hands of these investigators and as used later by the Winslows. The groups as found by such a procedure were found to agree in a large degree with the groups as determined by cultural methods.

Gordon again, in 1906, made an extensive survey of the white staphylococci which will be discussed more fully later. He studied approximately 300 cultures of cocci isolated from the skin and noted their reactions upon eight different media, but did not attempt any correlation of their results with the results of other workers who had used other strains.

In 1908, Dudgeon studied the relationships of a group of staphylococci with particular emphasis on the application of chromogenesis to the separation of the individual species. In addition to noting the pigments produced, he used the nine tests advocated by Gordon (1905) and, in addition, studied the opsonic index, agglutination reactions, etc. The fermentation of sugars was also included and the results of this extensive investigation seemed to indicate that the presence or absence of pigment played little part in the separation of species and that *Staphylococcus albus* and *S. aureus* were really one and each a variant of a central type. The Winslows (1908, p. 102) in commenting upon this work suggested that the tables of Dudgeon did not bear out his statements and that more careful study of his data indicated that differences were apparent between the orange strains and the non-pigment forms as shown in his published results.

Probably the most extensive and complete survey of the entire group of cocci, including the streptococci, etc., is that of the Winslows (1908). A large number of cultures were used and in general the larger groups (genera) of micrococci were formed on the basis of chromogenesis and habitat. The reduction of nitrates, liquefaction

of gelatin, and fermentation of certain sugars were used for the separation of species. From the standpoint of opening up the field and pointing out the larger relations of the group, this work is of great value and more detailed reference will be made to it later.

In 1913, Kligler repeated the work of Dudgeon. He used the cultures in the American Museum of Natural History Collection and found that his results did not agree with those of the earlier investigators in regard to the importance of pigment production in the separation of natural groups of the micrococci. From his observations he concluded that the white and orange types usually represented strains from parasitic sources and the yellow and red strains were more often saprophytic.

Marbias (1919) in attempting to find a diagnostic test of value in separating various strains of staphylococci decided that milk and gelatin served as ideal media for the differentiation of the members of this group and arranged various sub-groups of the micrococci based on their reactions with milk and the liquefaction of gelatin. Such a classification resulted in the formation of five sub-groups with each of the sub-groups designated as such and not given a definite species name. Other characters were also studied to aid in further differentiating the various groups.

Winslow, Rothberg, and Parsons (1920), in following out Kligler's suggestion that the saprophytic and parasitic forms could be separated by pigment production, studied intensively several members of the orange and white groups and divided the group into several species upon the basis of the production of acid from lactose and the liquefaction of gelatin.

Liquefaction of gelatin, action upon milk, and Gram stain were used by Kempel in 1920 in classifying 56 strains of micrococci after comparing a long series of tests. Serological tests also seemed to show promise of correlating with the cultural tests and so aiding in differentiating the various species.

Hine (1922) studied 90 strains of micrococci, all, however, isolated from parasitic sources, and separated his cultures by aggultination and agglutination-absorption tests into two groups.

Julianelle (1922) made a survey of several species of hemolytic staphylococci and found that several sub-groups could be formed on the basis of certain serological reactions.

These investigations include all of the more important surveys of the group in which a series of cultures were used. The preceding

paper of this series (Technical Bulletin No. 99) gives in greater detail the systems of classifications used by various observers in arranging the cocci, and the reader is referred to this review for a more complete history of our knowledge of the general classification of the Coccaceae. References are made later to work which deal with special points only.

ORIGIN AND SOURCE OF CULTURES

The cultures used in the following investigation were collected from various sources with the thought kept in mind that a representative series should be collected containing organisms from all habitats commonly inhabited by members of this group. Three hundred and sixteen cultures were used, 105 of which were secured from Johns Hopkins University; 79 from Parke, Davis and Co.; 9 from Lederle Antitoxin Laboratories; 7 from the Kral collection in Vienna; 9 from the National Collection of Type Cultures of the Lister Institute, London; 15 from the American Museum of Natural History Collection; 34 from various laboratories thruout the United States and Europe; and 58 were freshly isolated.

Of the 194 cultures isolated from human and mammalian bodies, 64 were endoparasitic and were obtained from pathologic lesions of various kinds, and 130 were found on the surface of similar bodies. While it is assumed that the cultures isolated from lesions are pathogenic, the organisms isolated were not definitely determined to be the etiological factor causing the lesion except in a few cases.

The 86 cultures isolated from saprophytic sources were derived as follows: 49 from milk and from dairy utensils, 8 from water, 11 from human and animal feces, 5 from soil, 3 from leather, 2 from the surface of ripe tomatoes, 3 from cheese, 3 from air, 1 from the surface of a potato, and 1 from putrid meat. The remaining 36 strains were received with only a name attached and no information could be secured as to their source, but in a large number of the cases they were probably non-parasitic types.

Table 1 gives in outline the general characters of the 316 organisms studied, together with the source of each strain. Any numerical system of recording results is subject to certain limitations. The system here presented has been slightly modified from that suggested by the Committee on Technic of the Society of American Bacteriologists in order to fit the present need. It serves as an excellent method of reproducing results in a condensed form. The key is given below:

KEY TO NUMERICAL SYSTEM USED IN RECORDING THE GENERAL CHARACTERS.

Microscopic Features		Form: 1, streptococci; 2, diplococci; 3, micrococci; 4, sarcinae; 5, rods; 6, commas; 7, spirals; 8, branched rods; 9, filamentous.
		Spores: 1, central; 2, polar; 3, absent.
		Flagella: 1, peritrichic; 2, polar; 3, absent.
		Gram stain: 1, positive; 2, negative; 3, variable; 4, variable generally positive; 5, variable generally negative.
Miscellaneous Biochemical Reactions		Habitat: 1, human lesion; 2, human ectoparasite; 3, animal lesion; 4, animal ectoparasite; 5, udder; 6, milk; 7, cheese; 8, water; 9, air; 0, miscellaneous saprophyte; X, unknown origin.
		Relation to oxygen: 1, strict aerobe; 0, facultative anaerobe; 2, microaerophilic; 3, strict anaerobe.
		Gelatin liquefaction: 1, positive; 2, negative.
		In nitrate media: 1, nitrite and gas; 2, nitrite but no gas; 3, neither nitrite nor gas.
		Chromogenesis: 1, fluorescent; 2, violet; 3, blue; 4, green; 5, yellow; 6, orange; 7, red; 8, brown; 9, pink; 0, none.
Carbohydrate Reactions		Diastatic action: 1, positive; 2, negative.
		From dextrose: 1, acid and gas; 2, acid without gas; 3, no acid.
		From lactose: 1, acid and gas; 2, acid without gas; 3, no acid.
		From sucrose: 1, acid and gas; 2, acid without gas; 3, no acid.
Cultural Features		Diameter: 1, under 0.5μ; 2, between 0.5μ and 1μ; 3, over 1μ.
		Growth on amino nitrogen: 1, positive; 2, negative.
		Ammonia production from peptone: 1, positive; 2, negative.
		Indol production: 1, positive; 2, negative.
		Ammonia production from nitrates: 1, growth with no NH_3; 2, growth with NH_3; 3, no growth.
		Hemolysis of blood: 1, positive; 2, negative.
	Agar Stroke	Abundance: 1, abundant; 2, moderate; 3, slight; 4, absent.
		Lustre: 1, glistening; 2, dull.
		Surface: 1, smooth; 2, contoured; 3, rugose.
		Agar colonies: 1, punctiform; 2, round (over 1 mm. diameter); 3, rhizoid; 4, filamentous; 5, curled.
		Gelatin colonies: 1, punctiform; 2, round (over 1 mm.); 3, irregular, 4, filamentous.
	Milk	Acid: 1, sufficient for curdling; 2, insufficient for curdling; 3, no acid.
		Rennet curd: 1, present; 2, absent.
		Peptonization; 1, present; 2, absent.
		Maximum H-ion concentration: 1, pH 7.0; 2, 7.0 to 6.0; 3, 6.0 to 5.0; 4, 5.0 to 4.0; 5, no growth.
		Growth on ammonia nitrogen: 1, positive; 2, negative.
		Nature of growth: 1, chalky; 2, transparent; 3, semi-cloudy; 4, chromogenic.
		Nature of cells: 1, even and regular; 2, uneven and irregular.
		Growth in broth: 1, cloudy; 2, precipitate; 3, no growth.

TABLE 1.—OUTLINE OF GENERAL CHARACTERS OF THE CULTURES OF MICROCOCCI USED IN THIS SURVEY.

No. of culture	Name attached when received*	Source	Form	Spores	Flagella	Gram	Habitat	Oxygen requirements	Gelatin liquefaction	Nitrate reduction	Chromogenesis	Diastatic action	Dextrose	Lactose	Sucrose	Diameter	Amino N utilization	NH_3 production from peptone	Indol	NH_3 production from NO_3	Hemolysis of blood	Agar stroke: Abundance	Agar stroke: Lustre	Agar stroke: Surface	Agar colony	Gelatin colony	Milk: Acid	Milk: Rennet	Milk: Peptonization	Maximum H-ion production	Ammonia N utilization	Nature of growth	Nature of cells	Growth in broth	Origin of culture†
1	*M. varians*		3	3	3	5	x	2	1	3	5	1	2	2	3	2	1	1	2	3	2	2	1	1	0	0	1	2	2	1	1	4	1	2	C. E. M.
2		Dairy utensils	3	3	3	4	6	0	1	2	5	1	2	2	3	2	1	1	2	1	2	2	1	1	0	0	3	1	1	4	1	0	1	1	G. J. H.
3		Milk	3	3	3	5	6	2	2	2	5	2	2	2	2	2	1	2	2	1	2	3	2	1	0	0	2	2	2	4	1	4	2	2	"
4		Milk	3	3	3	5	6	0	1	2	5	1	2	2	2	2	1	2	2	1	2	2	1	1	0	0	1	2	2	4	1	4	2	2	"
5		Skim milk	3	3	3	5	2	0	1	2	5	2	2	2	3	3	1	1	2	1	2	2	1	1	0	0	1	2	1	5	1	1	2	3	"
7	*Staph. citreus*		3	3	3	3	x	0	1	3	5	2	2	3	3	2	1	1	2	1	2	2	1	1	0	0	3	1	1	5	1	4	1	3	W. G.
8		Dairy utensils	3	3	3	5	6	0	1	3	5	2	2	2	3	2	1	1	2	1	2	2	1	1	0	0	2	2	2	4	1	4	1	2	A. H. R.
9		Skin, hand	3	3	3	4	2	0	1	3	5	2	2	2	3	2	1	1	2	1	2	1	1	1	0	0	1	2	1	5	1	4	1	3	G. J. H.
11		Unknown	3	3	3	5	x	1	0	3	5	2	2	2	2	2	1	1	2	1	2	2	1	1	0	0	3	1	1	5	1	4	1	3	"
13		Dairy utensils	3	3	3	4	6	0	1	2	5	2	2	2	3	2	1	1	2	1	2	2	1	1	0	0	1	2	2	4	1	4	1	2	A. H. R.
15		Milk	3	3	3	4	6	0	1	2	5	2	2	2	2	2	1	1	2	1	2	2	1	1	0	0	1	2	2	4	1	4	1	2	G. J. H.
17		Water	3	3	3	4	8	2	1	2	6	2	2	2	2	3	2	1	1	2	1	2	2	3	1	1	0	0	1	2	1	4	1	2	G. J. H.
16	*M. citreus*		3	3	3	4	x	0	1	3	5	2	2	3	3	2	1	1	2	1	2	2	1	1	0	0	1	2	1	4	1	4	1	2	A.M.N.H. No. 466
18	*M. flavus*		3	3	3	3	x	0	1	2	5	2	3	3	3	2	2	1	2	1	2	2	1	1	0	0	3	2	2	1	1	4	1	2	" No. 272
20		Water	3	3	3	3	8	0	1	2	5	2	2	2	2	2	1	1	2	1	2	2	1	1	0	0	1	2	2	1	1	4	1	2	G. J. H.
21		Unknown	3	3	3	3	x	0	1	2	5	2	2	2	2	2	1	1	2	2	2	3	1	1	0	0	1	2	2	4	1	2	1	2	"
22		Udder	3	3	3	4	5	0	1	2	5	2	2	2	3	2	2	1	2	2	2	2	1	1	0	0	1	2	2	5	1	4	1	3	W. A. W.
24		Udder	3	3	3	4	5	2	2	2	0	2	2	3	2	2	1	1	2	1	2	3	1	1	0	0	1	2	2	4	1	3	1	2	W. A. W.
25		Milk	3	3	3	5	6	2	1	2	5	2	2	2	2	2	1	1	2	1	2	3	1	1	0	0	1	2	1	4	1	4	1	2	A. H. R.
27		Milk	3	3	3	3	6	0	1	2	5	2	2	2	2	2	1	1	2	1	2	2	1	1	0	0	3	1	1	5	1	4	1	3	A. H. R.
28		Milk	3	3	3	3	6	0	1	3	5	1	2	2	2	2	1	1	2	2	2	3	1	1	0	0	1	2	2	4	1	4	1	2	G. J. H.

29	.....	Milk	3	3	3	3	6	0	1	2	5	2	2	2	2	2	1	1	2	1	2	2	1	1	0	0	1	2	2	4	1	4	1	2	G. J. H.
30	.....	Milk	3	3	3	4	6	0	1	2	5	2	2	2	2	2	1	1	2	2	2	2	1	1	0	0	2	2	2	4	1	4	2	2	"
32	.....	Milk	3	3	3	3	6	0	1	3	5	2	2	2	2	2	1	1	2	1	2	3	1	1	0	0	1	2	1	5	1	4	1	3	"
33	.....	Milk	3	3	3	3	6	0	1	2	5	2	2	2	2	2	1	1	2	2	2	2	1	1	0	0	1	2	2	4	1	4	1	1	"
34	.....	Milk	3	3	3	5	6	0	1	2	5	2	2	2	3	2	1	1	2	1	1	2	1	1	0	0	1	2	2	4	1	4	2	2	A. H. R.
36	.....	Milk	3	3	3	4	6	0	1	2	5	2	2	2	3	2	1	1	2	2	2	2	1	1	0	0	1	2	1	2	1	4	1	1	G. J. H.
39	*Staph. pyogenes. citreus*	.....	3	3	3	4	x	0	1	3	5	2	3	3	3	2	2	1	2	1	1	2	1	1	0	0	3	2	2	1	1	4	2	2	Kral
40	*M. pyogenes citreus*	.....	3	3	3	3	x	0	1	3	5	2	3	3	3	2	1	1	2	1	1	2	1	1	0	0	3	2	2	1	1	4	2	2	Kral
42	.....	Milk	3	3	3	5	6	0	1	2	5	2	2	2	3	2	1	1	2	2	2	2	1	1	0	0	1	2	1	4	1	4	1	1	G. J. H.
44	.....	Milk	3	3	3	3	6	0	1	2	5	2	2	2	3	2	1	1	2	2	2	2	1	1	0	0	1	2	1	4	1	4	1	2	"
45	.....	Milk	3	3	3	4	6	0	1	2	5	2	3	3	3	1	1	2	2	1	2	2	2	3	0	0	1	2	2	1	1	4	1	2	"
48	*M. kerinis*	.....	3	3	3	5	x	1	0	2	5	2	3	3	3	2	2	1	2	2	1	1	0	0	3	2	2	1	2	4	1	2	Kral		
50	.....	Burn of leg	3	3	3	5	2	2	1	2	6	2	2	2	2	2	2	1	2	1	1	3	2	1	0	0	1	2	2	4	2	4	1	1	S. B-J. No. 84
51	.....	Throat	3	3	3	4	2	0	1	3	0	2	2	2	2	2	1	1	2	1	2	2	1	1	0	0	3	1	2	4	1	3	1	2	" " 61
53	.....	Milk	3	3	3	5	6	0	1	2	5	2	2	2	3	2	1	1	2	1	2	2	1	1	0	0	3	1	1	4	1	4	1	2	G. J. H.
55	.....	Pneumonia	3	3	3	5	1	2	1	2	0	2	2	2	2	2	1	1	2	1	2	3	1	1	0	0	1	2	2	4	2	2	1	2	S. B-J. No. 15
56	.....	Milk	3	3	3	5	6	0	1	3	5	2	3	3	3	2	1	1	2	1	1	2	1	1	0	0	3	2	2	1	1	4	2	2	G. J. H.
57	.....	Milk	3	3	3	4	6	0	1	3	5	2	3	3	3	2	1	1	2	1	2	2	1	1	0	0	3	1	1	1	1	4	2	2	"
58	.....	Conjunctivitis	3	3	3	5	2	2	2	2	0	2	2	2	3	2	2	1	2	1	2	3	1	1	0	0	1	2	2	1	2	2	2	2	S. B-J. No. 71
59	.....	Infected thumb	3	3	3	5	2	2	2	2	0	2	2	2	2	2	2	1	2	1	2	3	2	1	0	0	1	2	1	4	2	2	1	2	" " 6
60	.....	Infection	3	3	3	4	2	2	1	2	0	2	2	2	2	2	2	1	2	1	2	2	2	1	0	0	1	2	1	4	2	2	1	2	" " 102
61	.....		3	3	3	5	2	0	2	2	5	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	2	4	2	4	1	2	" " 112
62	.....	Cheese	3	3	3	5	7	2	1	2	5	2	2	2	3	2	1	1	2	2	2	2	1	1	0	0	1	2	2	4	1	4	1	2	G. J. H.
63	.....	Infected finger	3	3	3	3	2	2	1	2	0	2	2	2	2	2	2	2	1	2	3	1	1	0	0	3	1	2	5	2	1	1	3	S. B-J. No. 22	
64	.....	Bullet wound	3	3	3	8	2	2	1	2	0	1	2	2	3	2	1	1	2	1	2	3	2	1	0	0	3	1	2	5	1	1	1	3	" " 28
65	.....	Milk	3	3	3	4	6	2	2	3	5	2	3	3	2	2	1	1	2	1	2	3	1	1	0	0	3	2	2	4	1	4	1	2	G. J. H.
67	.....	Milk	3	3	3	4	6	0	1	2	5	2	2	2	2	2	1	1	2	2	2	3	1	1	0	0	1	2	1	4	1	4	1	2	"
69	.....	Ulcer	3	3	3	4	2	0	1	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	1	4	2	2	1	2	S. B. J. No. 110
70	.....	Milk	3	3	3	3	6	0	1	2	5	1	2	3	3	2	1	1	2	2	2	1	1	0	0	1	2	1	1	1	4	1	2	G. J. H.	
71	*M. citreus*	.....	3	3	3	5	x	0	1	3	0	2	3	3	3	2	1	1	2	1	2	1	1	1	6	0	3	2	2	1	1	1	1	2	O-J.
72	*M. citreus*	.....	3	3	3	2	x	0	1	3	5	2	3	3	3	2	1	1	2	2	2	2	1	1	0	0	3	2	2	1	1	4	1	2	O-J.
73	.....	Milk	3	3	3	5	6	0	1	2	5	2	2	2	2	2	1	1	2	2	2	2	1	1	0	0	1	2	1	4	1	1	1	2	G. J. H.
74	.....	Milk	3	3	3	4	6	6	1	2	5	2	2	2	3	2	1	1	2	1	2	2	2	1	0	0	1	2	1	4	1	4	1	2	"
75	.....	Varicose ulcer	3	3	3	5	1	0	1	2	5	2	2	2	3	2	2	1	2	1	2	2	1	1	1	0	0	1	2	1	5	2	4	1	3	S. B-J. No. 92
76	.....	Milk	3	3	3	4	6	0	1	2	0	2	2	2	2	2	1	2	2	1	2	2	1	1	0	0	1	2	2	4	1	1	1	2	G. J. H.

TABLE 1.—OUTLINE OF GENERAL CHARACTERS OF THE CULTURES OF MICROCOCCI USED IN THIS SURVEY.—*Continued.*

No. of culture	Name attached when received*	Source	Form	Spores	Flagella	Gram	Habitat	Oxygen requirements	Gelatin liquefaction	Nitrate reduction	Chromogenesis	Diastatic action	Dextrose	Lactose	Sucrose	Diameter	Amino N utilization	NH_3 production from peptone	Indol	NH_3 production from NO_3	Hemolysis of blood	Agar stroke: Abundance	Agar stroke: Lustre	Agar stroke: Surface	Agar colony	Gelatin colony	Milk: Acid	Milk: Rennet	Milk: Peptonization	Maximum H-ion production	Ammonia N utilization	Nature of growth	Nature of cells	Growth in broth	Origin of culture†
78	*M. melitensis*		3	3	3	3	x	0	1	2	5	2	2	2	3	2	1	1	2	2	2	2	1	1	0	0	1	2	1	4	1	4	1	2	Kral
80		Water	3	3	3	1	8	0	2	2	5	2	2	2	3	2	1	1	2	2	2	2	1	1	0	0	1	2	2	4	1	4	1	2	G. J. H.
81		Water	3	3	3	4	8	0	1	2	5	1	2	2	2	2	1	1	2	1	2	2	1	1	0	0	1	2	2	4	1	4	1	2	"
84		Cheese	3	3	3	4	7	0	1	3	5	2	2	3	2	2	1	1	2	1	2	2	1	1	0	0	1	2	1	4	1	4	1	2	"
87		Udder	3	3	3	5	5	0	1	2	5	1	3	3	2	2	2	1	2	1	2	2	1	1	0	0	3	2	2	5	1	4	2	3	"
88		Soil	3	3	3	5	0	0	1	3	0	2	3	3	3	2	1	1	2	1	2	2	1	1	0	0	1	2	2	5	1	3	2	3	Conn 17.5 M 413.
90		Infected finger	3	3	3	3	2	2	1	2	0	2	2	2	2	2	1	1	2	1	2	3	1	1	0	0	1	2	2	4	2	4	1	2	S. B-J. No. 38
91		Throat	3	3	3	3	2	0	1	2	0	2	2	2	2	2	1	1	2	1	2	3	1	1	0	0	1	2	2	4	2	4	1	2	" " 64
92		Udder	3	3	3	3	5	0	1	2	5	1	3	3	3	2	1	1	2	1	2	2	1	1	0	0	3	2	2	1	1	4	2	2	G. J. H.
93		Udder	3	3	3	5	5	0	1	2	5	2	2	2	2	2	1	1	2	1	2	2	1	1	0	0	3	2	2	1	1	4	1	2	"
94		Throat	3	3	3	4	x	2	1	2	5	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	2	2	2	2	1	2	S. B-J.
95		Dairy utensils	3	3	3	4	6	0	1	2	5	2	2	2	3	2	1	1	2	1	2	1	1	1	0	0	2	2	2	4	1	4	1	2	A. H. R.
96		Dairy utensils	3	3	3	4	6	0	2	2	5	2	2	2	3	2	1	1	2	1	2	1	1	1	0	0	3	2	2	4	1	4	1	2	A. H. R.
97		Dairy utensils	3	3	3	3	6	0	1	2	5	2	2	2	3	2	1	1	2	1	2	1	1	1	0	0	1	2	2	5	1	4	1	3	G. J. H.
99		Infection	3	3	3	4	2	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	2	1	0	0	1	2	2	4	2	2	1	2	S. B-J. No. 20
100		Milk	3	3	3	4	6	0	1	3	5	2	2	2	3	2	1	1	2	1	2	2	1	1	0	0	1	2	1	1	1	4	1	2	G. J. H.
101		Milk	3	3	3	4	6	2	1	2	5	2	2	2	2	2	1	1	2	1	2	2	1	1	0	0	2	2	2	4	1	4	2	2	G. J. H.
102		Alveolar abscess	3	3	3	5	1	0	1	2	5	2	2	2	2	2	2	2	2	1	2	3	1	1	0	0	3	1	1	4	2	2	1	2	S. B-J. No. 107
105		Feces, human	3	3	3	5	0	0	1	2	5	2	2	2	2	2	1	2	2	1	2	3	1	1	6	0	1	2	1	4	2	2	1	2	" " 59
111		Putrid meat	3	3	3	4	0	0	1	2	0	1	2	2	3	2	1	2	2	2	2	2	2	1	0	0	1	2	1	2	1	1	1	2	" " 115
112			3	3	3	4	x	0	1	2	5	1	2	2	2	2	1	1	2	1	2	1	1	1	0	0	1	2	1	4	1	4	1	2	G. J. H.

113	Conjunctivitis	3	3	3	1	2	0	1	2	5	2	2	3	2	2	2	1	2	2	2	3	1	1	0	0	1	2	2	4	2	2	1	2	S. B-J. No. 85			
115	Deep skin	3	3	3	1	2	0	2	3	0	2	2	2	3	2	2	1	2	1	2	3	1	1	0	0	2	2	2	1	2	2	1	2	L. A. L.			
116	Deep skin	3	3	3	3	2	0	1	2	5	2	2	2	2	2	1	1	2	1	2	2	1	1	0	0	3	1	1	4	1	4	2	2	L. A. L.			
118	Deep skin	3	3	3	4	2	0	1	2	5	2	2	3	2	2	1	1	2	1	2	2	1	1	0	0	3	1	1	4	1	4	1	2	L. A. L.			
119	Deep skin	3	3	3	4	2	0	1	2	5	2	2	2	2	2	1	1	2	1	2	2	1	1	0	0	1	2	1	3	1	4	1	1	L. A. L.			
120	Deep skin	3	3	3	5	2	0	1	2	5	2	2	3	2	2	1	1	2	1	2	1	1	1	0	0	3	1	1	4	1	4	1	2	"			
121	Deep skin	3	3	3	5	2	2	2	2	0	2	2	2	2	2	1	1	2	1	2	2	1	1	0	0	3	2	2	3	1	1	1	2	"			
122	Deep skin	3	3	3	5	2	0	1	2	5	2	2	3	2	2	1	1	2	1	2	2	1	1	0	0	3	1	1	4	1	4	2	2	"			
123	Deep skin	3	3	3	3	2	2	1	2	0	2	2	2	2	2	1	2	2	1	2	3	2	1	0	0	1	2	1	4	1	2	1	2	"			
124	Deep skin	3	3	3	4	2	2	1	2	0	2	2	2	2	2	1	2	2	1	2	3	1	1	0	0	1	2	1	4	2	2	1	1	"			
125	Osteomyelitis	3	3	3	4	1	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	2	1	0	0	1	2	1	4	2	2	1	2	S. B-J. No. 81			
126	Boil	3	3	3	3	2	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	4	4	2	2	1	1	"	"	18	
127	Colon, human	3	3	3	4	0	2	1	2	0	2	2	3	2	2	2	1	2	1	2	3	1	1	0	0	3	1	1	4	2	2	1	2	"	"	58	
128	Infection of foot	3	3	3	5	2	0	1	2	5	2	2	2	3	2	1	1	2	1	2	2	1	1	0	0	1	2	1	4	2	4	1	2	"	"	78	
129	Air	3	3	3	4	9	0	1	2	0	2	2	2	2	2	1	1	2	1	2	2	1	1	0	0	1	2	2	3	1	1	1	2	"	"	66	
130	Varicose ulcer	3	3	3	5	1	2	1	2	0	2	2	2	2	2	2	1	2	1	2	2	1	1	0	0	1	2	1	3	2	1	1	2	"	"	77	
131	Abscess	3	3	3	4	1	2	1	2	5	2	2	2	2	2	2	1	2	2	2	2	1	1	0	0	1	2	1	4	2	4	1	2	"	"	74	
132	Infection	3	3	3	4	2	0	1	2	6	2	2	2	2	2	2	1	2	1	2	3	1	3	0	0	1	2	2	4	2	4	1	2	"	"	97	
133	Feces	3	3	3	4	0	2	1	2	5	2	2	2	2	2	1	1	2	1	2	3	1	1	0	0	1	2	1	4	1	4	1	1	"	"	56	
134	Infected wound	3	3	3	4	2	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	3	2	2	4	2	2	2	2	"	"	23	
135	Infected hand	3	3	3	4	2	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	1	4	2	2	1	1	"	"	26	
136	Infected foot	3	3	3	4	2	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	2	4	2	1	1	2	"	"	25	
137	Boil	3	3	3	5	2	2	1	2	6	2	2	2	2	2	2	1	2	2	2	2	1	1	0	0	1	2	2	4	2	4	1	2	G. J. H.			
138	Boil	3	3	3	4	2	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	2	1	0	0	1	2	1	4	2	2	1	2	S. B-J. No. 88			
139	Abscess of finger	3	3	3	4	1	2	1	2	5	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	2	4	2	2	1	2	"	"	36	
140	Wound	3	3	3	4	2	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	2	4	2	2	1	2	"	"	72	
141	Boil	3	3	3	3	2	2	1	2	0	2	2	3	2	2	2	1	2	1	2	3	1	1	0	0	3	1	1	4	2	2	1	1	"	"	1	
142	Sputum	3	3	3	1	2	2	2	3	0	2	2	2	2	2	2	2	2	2	2	3	1	1	0	0	3	2	2	3	2	2	2	1	"	"	62	
143	Paronychia	3	3	3	5	1	0	1	2	5	2	2	2	2	2	2	1	2	1	1	3	1	1	0	0	1	2	2	3	2	2	1	1	"	"	100	
144	Spleen	3	3	3	1	1	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	2	3	2	2	1	1	"	"	113	
145	Water	3	3	3	3	8	0	1	2	0	2	2	2	2	2	1	1	2	1	1	3	1	1	0	0	1	2	2	4	1	3	1	1	G. J. H.			
146	Varicose ulcer	3	3	3	3	1	2	1	2	5	1	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	2	1	2	4	2	1	S. B-J. No. 27			
147	Boil	3	3	3	3	2	0	1	2	5	2	2	2	3	2	1	1	2	1	2	2	1	1	0	0	3	1	1	1	1	4	2	1	"			
148	Infected wound	3	3	3	3	2	2	1	2	0	2	2	2	2	2	2	1	2	1	1	3	1	1	0	0	1	2	1	3	2	2	2	1	"	"	45	
149	Infection	3	3	3	3	2	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	1	4	2	2	1	1	"	"	109	
150	Wound	3	3	3	5	2	2	2	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	2	3	2	2	1	1	"	"	76	
151	Ingrown toe nail	3	3	3	5	2	2	1	2	0	2	3	3	3	2	2	2	1	2	1	2	3	1	1	0	0	1	2	2	3	2	2	1	"	"	108	

TABLE 1.—OUTLINE OF GENERAL CHARACTERS OF THE CULTURES OF MICROCOCCI USED IN THIS SURVEY.—*Continued.*

No. of culture	Name attached when received*	Source	Form	Spores	Flagella	Gram	Habitat	Oxygen requirements	Gelatin liquefaction	Nitrate reduction	Chromogenesis	Diastatic action	Dextrose	Lactose	Sucrose	Diameter	Amino N utilization	NH_3 production from peptone	Indol	NH_3 production from NO_3	Hemolysis of blood	Agar stroke: Abundance	Agar stroke: Lustre	Agar stroke: Surface	Agar colony	Gelatin colony	Milk: Acid	Milk: Rennet	Milk: Peptonization	Maximum H-ion production	Ammonia N utilization	Nature of growth	Nature of cells	Growth in broth	Origin of culture†
152	.....	Water.....	3	3	3	4	8	2	2	2	6	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	2	3	2	2	1	1	G. J. H.
153	.....	Water.....	3	3	3	4	8	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	2	1	0	0	1	2	2	1	2	2	2	1	" "
154	.....	Lung.....	3	3	3	3	1	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	1	4	2	2	2	1	S. B-J. No. 11
155	.....	Ulcer.....	3	3	3	3	1	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	1	3	2	2	2	1	" " 60
156	.....	Infection.....	3	3	3	5	2	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	3	2	2	4	2	2	2	1	" " 89
157	.....	Peritonitis.....	3	3	3	3	1	0	1	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	1	4	2	3	1	1	" " 12
158	.....	Septicemia.....	3	3	3	5	1	2	1	2	5	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	1	3	2	4	1	1	" " 8
159	.....	Varicose ulcer.....	3	3	3	4	1	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	2	4	2	3	1	1	" " 95
160	.....	Osteomyelitis.....	3	3	3	4	1	2	1	2	6	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	2	3	2	4	1	1	" " 4
161	.....	Abscess.....	3	3	3	4	1	2	2	3	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	2	4	2	2	2	1	" " 96
162	.....	Varicose ulcer.....	3	3	3	5	1	0	1	2	0	2	2	3	3	2	2	1	2	1	1	3	1	1	0	0	3	1	1	4	2	2	1	2	" " 46
163	.....	Cellulitis of hand.	3	3	3	4	2	0	1	2	0	2	2	2	2	2	2	1	2	1	1	3	1	1	0	0	1	2	2	4	2	3	1	2	" " 99
164	.....	Nasal discharge....	3	3	3	5	2	2	1	2	0	2	2	2	2	2	2	1	2	1	1	3	1	1	0	0	1	2	2	4	2	2	2	2	" " 111
165	.....	Abscess.....	3	3	3	5	1	2	1	2	0	2	2	2	2	2	2	1	2	1	1	3	1	1	0	0	1	2	2	4	2	2	1	2	" " 53
166	.....	Wound.....	3	3	3	4	2	2	1	2	0	2	2	2	2	2	2	1	2	1	1	3	1	1	0	0	1	2	2	4	2	2	1	2	" " 83
167	.....	Septicemia.....	3	3	3	4	1	2	1	2	5	2	2	2	2	2	2	1	2	1	1	3	1	1	0	0	1	2	2	4	2	2	1	1	" " 7
168	.....	Infection.....	3	3	3	4	2	2	1	2	0	2	2	2	2	2	2	1	2	1	1	3	1	1	0	0	1	2	1	4	2	2	2	2	" " 33
169	.....	Lungbroncho-pneu.	3	3	3	3	1	2	1	2	0	2	2	2	2	2	2	1	2	1	2	2	1	1	0	0	3	2	2	4	2	2	1	2	" " 13
172	.....	Cervical adenitis...	3	3	3	5	1	2	1	2	0	2	2	2	2	2	2	1	2	1	1	3	1	1	0	0	1	2	2	4	2	2	1	2	" " 105
173	.....	Cellulitis.....	3	3	3	4	2	2	1	2	0	2	2	2	2	2	2	1	2	1	1	3	1	1	0	0	1	2	2	4	2	2	1	2	" " 50
174	.....	Throat.....	3	3	3	4	2	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	1	1	2	2	1	2	" " 65

175	Wound	3	3	3	4	2	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	1	4	1	2	1	2	S. B-J.	No.	93
176	Infection	3	3	3	4	1	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	2	4	2	2	1	2	"	"	87
178	Infected foot	3	3	3	5	2	2	1	2	0	2	2	2	3	2	2	1	2	1	2	3	2	1	0	0	1	2	1	4	2	3	1	2	"	"	19
179	Wound of arm	3	3	3	5	2	2	1	2	6	2	2	2	2	2	2	1	1	1	2	2	1	1	0	0	1	2	2	5	2	2	1	3	"	"	16
170	Infection	3	3	3	5	1	2	1	2	5	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	2	4	2	2	1	2	S. B-J.	No.	44
180	Abscess of face	3	3	3	4	2	2	1	2	5	2	2	2	2	2	2	1	2	1	2	3	2	1	0	0	1	2	2	1	2	2	1	2	"	"	37
181	Abscess of kidney	3	3	3	5	1	2	1	2	0	2	2	2	2	2	2	1	2	1	1	3	2	1	0	0	1	2	2	4	2	2	1	2	"	"	9
182	Feces, human	3	3	3	5	0	2	1	2	0	2	2	2	2	2	2	1	2	1	1	3	1	1	0	0	1	2	2	4	2	2	1	2	"	"	54
183	Infected finger	3	3	3	5	2	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	2	4	2	2	1	2	"	"	3
184	Infected hand	3	3	3	4	2	2	1	2	5	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	2	4	2	2	1	2	"	"	31
185	Infection	3	3	3	4	2	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	2	4	2	2	1	2	"	"	10
186	Abscess of kidney	3	3	3	4	1	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	2	4	2	2	1	2	"	"	52
187	Infected finger	3	3	3	5	2	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	2	4	2	2	1	2	"	"	39
188	Ulcer	3	3	3	4	1	0	2	2	0	2	2	2	2	2	2	1	2	1	2	2	1	1	0	0	2	2	2	4	2	1	1	2	"	"	34
189	Varicose ulcer	3	3	3	4	1	2	1	2	5	2	2	2	2	2	2	1	2	1	2	3	1	3	0	0	1	2	2	4	2	4	1	1	"	"	80
190	Infection	3	3	3	5	2	0	2	2	0	2	2	2	2	2	2	2	1	1	2	3	1	1	0	0	2	1	1	4	2	2	2	2	"	"	40
191	Ulcer	3	3	3	4	1	0	2	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	2	2	2	4	2	2	2	1	"	"	5
192	Infection	3	3	3	4	2	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	2	4	2	2	2	1	2	"	"	29
193	Feces, human	3	3	3	4	0	0	1	3	5	2	2	2	2	2	1	1	2	1	1	2	1	1	0	0	3	1	1	4	1	4	2	2	"	"	57
194	Ulcer of skin	3	3	3	1	2	0	2	2	0	2	2	2	2	3	1	1	2	1	2	3	1	1	0	0	2	2	2	4	1	1	2	2	"	"	43
195	Infection	3	3	3	3	2	2	1	2	5	1	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	1	4	2	2	2	2	"	"	42
196	Water	3	3	3	5	8	6	1	3	5	2	3	3	3	2	2	1	2	1	1	2	1	1	0	0	3	2	2	4	2	4	2	2	G. J. H.		
197	Feces, human	3	3	3	3	0	0	1	2	7	2	2	2	3	2	2	1	2	1	2	2	1	1	0	0	3	2	2	1	1	4	2	1	S. B-J.	No.	57
198	Cellulitis of finger	3	3	3	5	2	2	1	2	5	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	2	4	2	2	1	2	"	"	106
199	Adenitis	3	3	3	4	1	2	1	2	0	2	2	2	2	2	2	1	2	1	1	3	1	1	0	0	1	2	2	4	2	2	1	1	2	G. J. H.	
200	Abscess	3	3	3	4	1	2	1	2	0	2	2	2	3	2	2	1	2	1	1	3	1	1	0	0	1	2	1	4	2	2	2	1	1	S. B-J.	No.	103
201	Cellulitis	3	3	3	3	2	2	1	2	0	2	2	2	2	2	2	1	2	1	1	3	1	1	0	0	1	2	2	4	2	2	2	1	1	S. B-J.	No.	75
202	Infected finger	3	3	3	4	2	2	1	2	5	2	2	2	2	2	2	1	2	1	2	2	1	1	0	0	1	2	1	4	2	3	1	2	"	"	82
203	Infection	3	3	3	4	2	2	1	2	0	2	2	2	2	2	2	1	2	2	2	3	1	1	0	0	1	2	2	4	2	4	1	2	"	"	21
204	Infection of hand	3	3	3	5	2	0	1	2	5	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	3	1	2	4	2	2	1	1	"	"	79
205	Boil	3	3	3	4	2	0	1	2	0	2	2	2	2	2	2	1	2	1	1	3	1	1	0	0	1	2	1	4	2	2	1	2	"	"	14
206	Paronychia	3	3	3	4	1	2	1	2	5	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	2	4	2	2	1	2	"	"	41
207	Ulcer	3	3	3	5	1	2	2	2	5	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	2	2	2	4	2	2	1	1	"	"	98
208	Varicose ulcer	3	3	3	4	1	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	2	2	2	4	2	2	1	2	"	"	94
209	Infection of foot	3	3	3	4	2	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	1	4	2	2	1	2	G. J. H.		
210	Wound	3	3	3	4	2	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	2	2	2	4	2	2	1	2	S. B-J.	No.	103
211	Wound infection	3	3	3	4	2	2	1	2	5	1	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	1	4	2	4	1	2	"	"	32

TABLE 1.—OUTLINE OF GENERAL CHARACTERS OF THE CULTURES OF MICROCOCCI USED IN THIS SURVEY.—*Continued.*

No. of culture	Name attached when received*	Source	Form	Spores	Flagella	Gram	Habitat	Oxygen requirements	Gelatin liquefaction	Nitrate reduction	Chromogenesis	Diastatic action	Dextrose	Lactose	Sucrose	Diameter	Amino N utilization	NH_3 production from peptone	Indol	NH_3 production from NO_3	Hemolysis of blood	Agar stroke: Abundance	Agar stroke: Lustre	Agar stroke: Surface	Agar colony	Gelatin colony	Milk: Acid	Milk: Rennet	Milk: Peptonization	Maximum H-ion production	Ammonia N utilization	Nature of growth	Nature of cells	Growth in broth	Origin of culture†
212	.....	Boil.....	3	3	3	4	2	0	1	2	5	1	2	2	3	2	1	1	2	1	2	2	1	1	0	0	3	1	1	4	2	4	2	2	S. B-J. No. 30
213	.....	Feces, human.....	3	3	3	3	0	0	2	2	7	1	2	2	2	2	2	1	2	1	2	2	1	1	0	0	3	2	2	4	1	4	2	2	" " 55
214	.....	Tubercular sinus...	3	3	3	4	1	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	2	4	2	2	1	2	" " 35
215	.....	Greenhouse air....	3	3	3	3	9	0	1	2	0	1	2	2	2	2	1	1	2	1	2	3	1	1	0	0	1	2	2	4	1	2	1	2	G. J. H.
216	.....	Cervical adenitis...	3	3	3	1	1	2	1	2	0	2	2	2	2	2	2	1	2	1	2	2	1	1	0	0	1	2	1	4	2	4	1	2	S. B-J. No. 48
217	.....	Abscess.....	3	3	3	1	1	2	1	2	0	1	2	2	2	2	2	1	2	1	1	3	1	1	0	0	1	4	2	2	1	2	1	2	" " 96
218	.....	Infected foot.....	3	3	3	4	2	2	2	2	0	2	2	2	3	2	2	1	2	1	2	3	1	1	0	0	1	2	1	4	2	2	1	2	" " 49
221	.....	Boil.....	3	3	3	4	2	2	2	2	0	2	2	2	2	2	1	1	2	1	2	3	1	1	0	0	1	2	2	4	2	2	1	2	" " 104
223	.....	Boil.....	3	3	3	4	2	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	1	4	2	2	2	2	" " 86
224	.....	Air.....	3	3	3	4	9	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	1	4	2	2	1	2	" " 67
226	.....	Feces, bovine.....	3	3	3	2	0	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	2	2	2	5	2	2	2	3	G. J. H.
227	.....	Feces, horse.....	3	3	3	4	0	0	1	2	5	2	3	3	3	2	1	1	2	1	2	2	1	1	0	0	3	2	2	5	1	4	2	3	Conn 161B: 8
228	.....	Feces, horse.....	3	3	3	5	0	0	1	2	0	2	3	3	2	2	1	1	2	1	2	2	1	1	0	0	3	2	2	5	1	1	2	3	Conn 161B: 16
229	*Staph. albus*....	Pus.....	3	3	3	5	1	0	1	2	0	2	2	2	2	2	1	1	2	1	2	2	1	1	0	0	1	2	1	3	2	1	1	2	P. D. No. .01439
230	*Staph. albus*....	Snuffles, rabbit....	3	3	3	4	4	2	2	2	0	2	2	2	2	2	2	1	2	1	2	2	1	1	0	0	3	2	2	4	2	1	1	2	" " .01344
231	*Staph. albus*....	Snuffles, rabbit....	3	3	3	4	4	0	1	2	0	2	2	2	2	2	1	1	2	1	2	3	1	1	0	0	1	2	2	4	2	2	1	1	" " .0322
232	*Staph. albus*....	Pus.....	3	3	3	3	3	0	2	2	0	2	2	2	2	2	2	1	2	1	1	3	1	1	0	0	1	2	1	4	2	1	1	2	" " .01426
233	*Staph. albus*....	Unknown.....	3	3	3	4	3	0	1	2	0	2	2	3	2	2	2	1	2	1	1	2	1	1	0	0	1	2	2	3	2	2	2	2	" " .01236
234	*Staph. albus*....	Pus.....	3	3	3	4	3	0	1	2	0	2	2	2	2	2	2	1	2	1	1	2	1	1	0	0	1	2	1	4	2	1	2	2	" " .01440
235	*Staph. albus*....	Distemper.....	3	3	3	1	4	2	1	2	0	2	3	2	2	2	2	1	2	1	1	3	1	1	0	0	1	2	1	4	2	2	1	2	" " .0441
236	*Staph. albus*....	Influenza.....	3	3	3	1	4	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	1	4	2	2	1	1	" " .0471

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TABLE 1.—OUTLINE OF GENERAL CHARACTERS OF THE CULTURES OF MICROCOCCI USED IN THIS SURVEY.—*Continued.*

No. of culture	Name attached when received*	Source	Form	Spores	Flagella	Gram	Habitat	Oxygen requirements	Gelatin liquefaction	Nitrate reduction	Chromogenesis	Diastatic action	Dextrose	Lactose	Sucrose	Diameter	Amino N utilization	NH_3 production from peptone	Indol	NH_3 production from NO_3	Hemolysis of blood	Agar stroke: Abundance	Agar stroke: Lustre	Agar stroke: Surface	Agar colony	Gelatin colony	Milk: Acid	Milk: Rennet	Milk: Peptonization	Maximum H-ion production	Ammonia N utilization	Nature of growth	Nature of cells	Growth in broth	Origin of culture†
275	*Staph. albus*	Pus	3	3	3	4	3	2	1	2	6	2	2	2	2	2	1	1	2	1	2	3	2	1	0	0	1	2	1	4	2	2	1	1	P. D. No. .01442
276	*Staph. albus*	Impetigo contagiosa	3	3	3	1	1	2	1	2	6	1	2	2	2	2	2	1	2	3	2	3	1	1	0	0	2	2	2	4	2	2	1	1	" " " .0171
277	*Staph. albus*	Pus	3	3	3	4	4	2	2	2	0	1	2	2	2	2	2	1	2	3	2	3	2	3	0	0	1	2	2	4	2	2	1	1	" " " .0551
278	*Staph. albus*	Canine infection	3	3	3	4	1	2	1	2	5	2	2	2	2	2	2	1	2	1	2	2	1	1	0	0	1	2	2	4	2	2	2	1	" " " .01170
279	*Micro. catarrhalis*	Tonsil	3	3	3	1	2	2	2	2	0	2	2	2	2	2	2	1	2	1	2	2	2	1	0	0	3	2	2	4	2	2	1	1	" " " .01562
282	.....	Milk	3	3	3	5	6	2	1	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	3	2	2	4	2	2	1	2	G.J. H.
283	*Staph. albus*	Wound infection	3	3	3	1	4	2	1	2	0	2	2	3	2	2	2	1	2	1	2	3	1	1	0	0	1	2	2	4	2	2	2	2	P. D. No. .0555
284	*Staph. albus*	Pus	3	3	3	4	2	0	1	2	6	2	2	2	2	2	2	1	2	1	2	2	2	1	0	0	1	2	2	4	2	2	2	1	" " " .01498
285	*Staph. albus*	Boil	3	3	3	4	2	0	1	2	6	2	2	2	2	2	2	1	2	1	2	2	2	1	0	0	2	2	2	4	2	2	1	1	" " " .01299
286	*Staph. albus*	Throat	3	3	3	5	2	0	1	2	6	2	2	2	2	2	2	1	2	1	2	3	2	1	0	0	1	2	2	4	2	2	2	1	" " " .01377
287	*Staph. albus*	Pus	3	3	3	5	2	0	1	2	6	2	2	2	2	2	2	1	2	1	2	3	2	1	0	0	1	2	2	4	2	2	2	1	" " " .01508
288	*Staph. albus*	Boil	3	3	3	5	2	0	2	2	0	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	1	4	2	2	2	1	" " " .0394
289	*Staph. albus*	Pneumonia	3	3	3	5	1	2	1	2	6	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	2	4	2	2	2	1	" " " .01510
290	*Staph. albus*	Pus	3	3	3	4	2	0	1	2	6	2	2	2	2	2	2	1	2	3	2	3	1	1	0	0	2	2	2	4	2	2	2	1	" " " .0152
291	*Staph. aureus*	Pus	3	3	3	5	1	2	1	2	6	2	2	2	2	2	2	1	2	1	2	3	2	1	0	0	1	2	2	4	2	2	2	1	" " " .01499
292	*Staph. aureus*	Pus	3	3	3	5	2	2	1	2	6	2	2	2	2	2	2	1	2	3	2	3	2	1	0	0	1	2	2	4	2	2	2	2	" " " .01509
293	*Staph. aureus*	Urethritis	3	3	3	4	2	0	1	2	6	2	2	2	2	2	2	1	2	3	2	3	2	1	0	0	1	2	2	4	2	2	1	1	" " " .0316
294	*Staph. aureus*	Influenza	3	3	3	4	1	0	1	2	6	2	2	2	2	2	2	1	2	3	2	3	2	1	0	0	1	2	2	4	2	2	1	1	" " " .01378
295	*Staph. aureus*	Infection	3	3	3	4	2	0	1	2	6	2	2	2	2	2	2	1	2	3	2	3	2	1	0	0	1	2	2	4	2	2	1	2	" " " .01519
296	*Staph. aureus*	Whooping cough	3	3	3	4	2	2	1	2	6	2	2	2	2	2	1	1	2	3	2	3	1	1	0	0	2	2	2	4	2	2	1	2	" " " .0589

297	*Staph. aureus*	Pus	3	3	3	4	2	0	1	2	6	2	2	2	2	2	2	1	2	3	2	2	1	1	0	0	1	2	2	4	2	2	1	1	P. D. No.	.01452
298	*Staph. aureus*	Pus	3	3	3	4	2	0	1	2	6	2	2	2	2	2	2	1	2	3	2	3	1	1	0	0	1	2	2	4	2	2	1	1	" "	.01399
299	*Staph. aureus*	Influenza	3	3	3	5	1	0	1	2	6	2	2	2	2	2	2	1	2	3	2	3	1	1	0	0	1	2	2	4	2	2	1	1	" "	.01382
300	*Staph. aureus*	Influenza	3	3	3	4	1	2	1	2	6	2	2	2	2	2	2	1	2	3	2	3	2	1	0	0	1	2	2	4	2	2	1	1	" "	.01381
301	*Staph. aureus*		3	3	3	4	x	0	2	2	0	2	2	2	2	2	1	1	2	3	2	2	1	1	0	0	1	2	1	4	1	1	2	2	A. M. N. H.	No526
302	*Albococcus ureae.*		3	3	3	4	x	0	2	3	0	2	2	2	3	2	1	1	2	1	2	2	1	1	0	0	2	2	2	4	1	1	1	2	" "	464
303	*Albococcus epidermidis*		3	3	3	1	x	0	2	2	0	2	2	3	2	2	2	1	2	3	2	3	1	1	0	0	3	2	2	4	2	2	2	2	" "	263
304	*Staph. aurantiacus*		3	3	3	1	x	0	1	2	6	2	2	2	2	2	1	1	2	1	2	3	1	1	0	0	1	2	1	4	1	2	1	1	" "	348
305	*Staph. epidermidis*		3	3	3	1	x	0	1	3	0	2	2	3	3	2	2	1	2	2	2	2	1	1	0	0	3	2	2	4	2	1	2	2	" "	25a
306	*Stpha. candicans*		3	3	3	1	x	0	2	2	0	2	2	3	3	2	1	1	2	3	2	2	1	1	0	0	3	2	2	4	1	1	1	2	" "	261
307	*Staph. aureus*		3	3	3	1	x	0	1	2	6	2	2	2	2	2	1	2	2	1	2	3	1	1	0	0	2	2	2	4	1	2	1	2	" "	279
308	*Staph. tetragenus*		3	3	3	1	x	0	2	2	0	2	2	2	2	2	1	1	2	3	2	3	1	1	0	0	1	2	2	4	1	2	2	2	" "	209
309	*Staph aurantiacus*		3	3	3	5	x	0	1	2	6	2	2	2	2	2	1	1	2	3	2	1	1	1	0	0	1	2	1	4	2	1	2	2	" "	313
310		Milk	3	3	3	2	6	0	2	3	5	2	2	3	2	2	2	1	2	1	2	1	1	1	0	0	3	2	2	4	1	4	2	2	G. J. H.	
311	*Staph. aureus*		3	3	3	5	x	0	1	2	6	2	2	2	2	2	1	1	2	1	2	1	1	1	0	0	1	2	1	1	1	4	2	2	A. M. N. H.	No. 457c
312	*Staph. ureae*		3	3	3	4	x	0	1	3	0	2	2	3	2	2	1	1	2	3	2	2	1	1	0	0	2	2	2	4	2	1	2	2	" "	130
314	*Staph. candidus.*		3	3	3	1	x	0	2	3	0	2	2	3	2	2	1	1	2	1	2	2	1	1	0	0	3	2	2	4	2	1	2	2	" "	49a
315		Milk	3	3	3	1	6	0	1	2	5	2	2	2	2	2	1	1	2	3	2	2	1	1	0	0	2	2	2	4	1	2	2	2	G. J. H.	
318		Soil	3	3	3	2	0	0	1	3	0	2	3	2	2	2	1	1	2	1	2	3	1	1	0	0	3	2	2	1	1	2	2	2	Conn 3 Ail	
310		Milk	3	3	3	5	6	0	1	2	5	2	2	2	2	2	2	1	2	3	2	2	1	1	0	0	2	2	2	4	2	2	2	2	G. J. H.	
322		Milk	3	3	3	4	6	0	1	3	6	2	3	3	3	2	1	1	2	1	2	2	1	1	0	0	3	1	1	1	1	2	2	2	"	
323	*Micro. candicus.*		3	3	3	4	x	0	1	2	0	2	2	3	2	2	1	1	2	3	2	2	1	1	0	0	3	2	2	4	1	2	2	2	W. W.	
325	*Micro. candicus*		3	3	3	1	x	0	1	2	0	2	2	3	2	2	1	1	2	1	2	2	1	1	0	0	1	2	1	1	1	2	2	2	W. W.	
326	*Micro. dermidus.*		3	3	3	1	x	0	2	3	0	2	3	3	2	2	1	1	2	3	2	2	1	1	0	0	3	2	2	4	2	1	1	2	A. M. N. H.	No. 25a
328	*Micro. varians*	Dairy utensils	3	3	3	4	6	0	1	2	0	2	2	2	2	2	1	1	2	3	2	2	1	1	0	0	2	2	2	4	1	2	2	2	W. W.	
320		Condensed milk	3	3	3	4	6	0	1	3	0	2	2	2	2	2	2	1	2	3	2	3	1	1	0	0	1	2	1	4	1	2	2	2	P. Downs.	
336	*tetragenus*		3	3	3	1	x	2	2	2	0	2	2	2	2	2	1	1	2	3	2	2	1	1	0	0	1	2	2	4	1	4	2	2	" "	209
337			3	3	3	5	x	0	1	2	0	2	2	2	2	2	1	1	2	1	2	2	1	1	0	0	1	2	1	4	1	2	2	2	" "	
339		Udder	3	3	3	1	5	2	2	2	0	2	2	2	3	2	2	1	2	3	2	3	1	1	0	0	2	2	2	4	2	2	2	2	W. A. W.	
341	*Micro aurantiacus*		3	3	3	5	x	0	1	2	6	2	2	2	2	2	1	1	2	1	2	3	1	1	0	0	1	2	1	4	2	2	2	2	B. H. No.	157

TABLE 1—OUTLINE OF GENERAL CHARACTERS OF THE CULTURES OF MICROCOCCI USED IN THIS SURVEY.—*Continued.*

No. of culture	Name attached when received*	Source	Form	Spores	Flagella	Gram	Habitat	Oxygen requirements	Gelatin liquefaction	Nitrate reduction	Chromogenesis	Diastatic action	Dextrose	Lactose	Sucrose	Diameter	Amino N utilization	NH_3 production from peptone	Indol	NH_3 production from NO_3	Hemolysis of blood	Agar stroke: Abundance	Agar stroke: Lustre	Agar stroke: Surface	Agar colony	Gelatin colony	Milk: Acid	Milk: Rennet	Milk: Peptonization	Maximum H-ion production	Ammonia N utilization	Nature of growth	Nature of cells	Growth in broth	Origin of culture†
342	*Staph. aureus*		3	3	3	4	x	2	1	2	6	2	2	2	2	2	1	1	2	3	2	2	1	1	0	0	1	2	1	4	1	1	2	2	B. H. No. 129
343		Milk	3	3	3	5	6	2	1	2	5	2	2	2	2	2	1	1	2	1	2	3	1	1	0	0	2	2	2	4	1	4	2	2	G. J. H.
344	*Staph. aureus*		3	3	3	5	x	2	1	2	6	2	2	2	2	2	2	1	2	3	2	3	1	1	0	0	1	2	2	4	2	2	1	2	Kral
346	*Staph. auranti-acus*		3	3	3	5	x	0	1	2	6	2	2	2	3	2	1	1	2	3	2	1	1	1	0	0	2	2	2	4	1	1	2	2	Kral
348		Milk	3	3	3	4	6	0	1	2	5	2	2	2	2	2	1	1	2	1	2	2	1	3	0	0	1	2	1	4	1	2	2	2	G. J. H.
349			3	3	3	5	x	0	2	2	7	2	3	3	2	2	2	1	2	3	2	2	1	1	0	0	1	2	1	4	2	4	2	2	Kral
354		Udder	3	3	3	1	5	2	1	2	0	2	2	2	2	2	2	1	2	3	2	2	1	1	0	0	3	2	2	4	2	2	2	2	G. J. H.
355	*Staph. aureus*	Pus	3	3	3	4	1	2	1	2	6	2	2	2	2	2	2	1	2	3	2	2	2	1	0	0	1	2	1	3	2	2	2	2	P. D. No. .01510
356	*Staph. aureus*	Lung	3	3	3	4	1	2	1	2	5	2	2	2	2	2	2	1	2	3	2	3	1	1	0	0	1	2	2	4	2	2	1	1	" " .01380
357	*Staph. aureus*	Pus	3	3	3	4	1	2	1	2	6	2	2	2	2	2	2	1	2	3	2	2	1	1	0	0	1	2	1	2	2	2	2	2	" " .0992
358	*Staph. aureus*	Pus	3	3	3	4	1	0	1	2	6	2	2	2	2	2	2	1	2	3	2	3	1	1	0	0	1	2	1	4	2	2	2	2	" " .01450
359		Pus	3	3	3	5	2	0	1	2	0	2	2	2	2	2	2	1	2	3	2	3	1	1	0	0	1	2	2	4	2	1	2	2	P. D. No. .0959
360	*Staph. aureus*	Vagina	3	3	3	4	2	2	1	2	6	2	2	2	2	2	2	1	2	3	2	3	1	1	0	0	1	2	2	4	2	2	2	1	" " .0317
361	*Staph. aureus*	Ozena	3	3	3	4	1	2	1	2	6	2	2	2	2	2	2	1	2	3	2	3	1	1	0	0	1	2	1	4	2	2	1	1	" " .0842
362	*Staph. aureus*	Pyorrhea	3	3	3	4	2	2	1	2	6	2	2	2	2	2	2	1	2	2	3	3	1	1	0	0	1	2	1	4	2	2	1	2	P. D. No. .01052
363	*Staph. aureus*	Acne	3	3	3	4	2	0	1	2	6	2	2	2	2	2	2	1	2	3	2	3	1	1	0	0	1	2	2	4	2	2	2	1	" " .01162
364	*Staph. aureus*	Boil	3	3	3	5	2	0	1	2	6	2	2	2	2	2	2	1	2	3	2	3	1	1	0	0	1	2	2	4	2	2	2	1	P. D. No. .01281
365	*Staph. aureus*	Infected toe	3	3	3	4	2	2	1	2	6	2	2	2	2	2	2	1	2	3	2	3	1	1	0	0	1	2	1	4	2	1	2	1	" " .01239

366	.....	Pasteurized milk...	3	3	3	4	6	2	2	3	0	2	3	3	2	2	2	1	2	3	2	3	1	1	0	0	3	2	2	4	2	1	2	1	Phipps		
367	.....	Pasteurized milk...	3	3	3	4	6	2	1	2	5	2	2	2	2	2	2	1	2	3	2	3	1	1	0	0	1	2	2	4	2	2	2	1	Phipps		
368	.....	Pasteurized milk...	3	3	3	3	6	2	1	2	5	2	2	2	2	2	1	1	2	3	2	3	1	1	0	0	1	2	2	4	1	4	2	1	Phipps		
369	.....	Pasteurized milk...	3	3	3	5	6	2	1	2	5	2	2	2	2	2	1	1	2	3	2	2	1	1	0	0	1	2	2	4	1	4	2	1	Phipps		
370	.....	Pasteurized milk...	3	3	3	2	6	2	2	3	5	2	3	3	2	2	2	1	2	1	2	3	1	1	0	0	3	2	2	4	2	1	2	1	W. D.		
371	.....	Tomato catsup....	3	3	3	2	0	2	2	2	0	2	3	3	2	2	2	1	2	1	2	3	1	1	0	0	3	2	2	3	2	2	2	1	Dar.		
372	.....	Tomato catsup....	3	3	3	2	0	2	2	2	5	2	2	2	2	2	1	1	2	1	2	3	1	1	0	0	3	2	2	4	1	4	2	1	"		
373	.....	Pasteurized milk...	3	3	3	4	6	2	2	2	5	2	2	2	2	2	2	1	2	1	2	3	1	1	0	0	1	2	2	4	1	4	2	1	G. J. H.		
374	.....	Pasteurized milk...	3	3	3	3	6	0	2	2	5	2	2	2	3	2	2	1	2	1	2	2	1	1	0	0	3	2	2	4	2	4	2	1	"		
375	*Micro. caromatus*.....	Feces.....	3	3	3	4	0	0	2	2	5	2	3	3	2	2	1	1	2	1	2	2	1	1	0	0	2	2	2	3	1	2	2	1	N. C. T. C. No.		1111
376	*Micro. badius*...	Soil.....	3	3	3	3	0	0	1	3	5	2	2	2	2	2	2	1	2	2	2	2	1	1	0	0	2	2	2	1	2	4	2	1	"	"	1112
377	*Micro. flavus*....	Feces.....	3	3	3	3	0	0	1	2	5	2	3	3	2	2	2	1	2	1	2	1	1	1	0	0	3	2	2	1	1	4	2	1	"	"	1113
378	*Micro. roseus*....	Soil.....	3	3	3	3	0	0	1	2	7	2	3	3	2	2	2	1	2	3	2	1	1	1	0	0	2	2	2	1	1	4	2	1	"	"	1114
379	*Micro. sulphureus*.....	Milk.....	3	3	3	4	6	0	1	2	5	2	2	2	2	2	1	1	2	1	2	1	1	1	0	0	3	2	2	3	1	4	2	1	"	"	1115
380	"Yellow coccus"	.....	3	3	3	5	0	0	1	2	5	2	3	3	3	2	1	1	2	1	2	1	1	1	0	0	3	2	2	1	1	4	2	1	"	"	1152
381	"Yellow coccus"	.....	3	3	3	5	0	0	1	3	5	2	3	3	3	2	1	1	2	1	2	1	1	1	0	0	3	2	2	1	1	4	2	1	"	"	1153
382	"Orange coccus".	.....	3	3	3	3	0	0	1	2	6	1	2	3	2	2	2	1	2	3	2	3	1	1	0	0	3	2	2	3	2	4	2	1	"	"	1154
383	"White coccus".	.....	3	3	3	3	0	0	2	3	0	2	3	3	3	2	2	1	2	3	2	1	1	1	0	0	2	2	2	1	2	1	2	1	"	"	1155

*The author by including the above names in the table does not endorse in any way their validity, but merely notes a record of the name attached to the particular strain when received for study.

†Key to abbreviations:

C. E. M.: Dr. Chas. E. Marshal, Mass. Agric. College, Amherst, Mass.
W. G.: Dr. Ward Giltner, Mich. Agric. College, East Lansing, Mich.
A. H. R.: A. H. Robertson, N. Y. Agric. Exp. Sta., Geneva, N. Y.
A. M. N. H.: American Museum of Natural History, New York City.
Conn: Dr. H. J. Conn, N. Y. Agric. Exp. Sta., Geneva, N. Y.
W. W.: Dr. W. A. Whiting, Cornell University, Ithaca, N. Y.
B. H.: Dr. B. W. Hammer, Iowa State College, Ames, Iowa.
Phipps: Dr. W. H. Phipps, Missouri Dairy Co., Kansas City, Mo.
Dar.: Dr. C. A. Darling, Allegheny Coll., Meadville, Penna.
W. D.: W. D. Dotterrer, Bowman Dairy Co., Chicago, Ill.
W. A. W.: W. A. Wall, City Bacteriologist, Geneva, N. Y.
Kral: Kral Collection of Type Cultures, Vienna, Austria.
S. B-J.: S. Bayne-Jones, Johns Hopkins Univ., Baltimore, Md.
O-J.: Dr. S. Orla-Jensen, Copenhagen, Denmark.
L. A. L.: Lederle Antitoxin Lab., Pearl River, N. Y.
P. Downs: Dr. P. A. Downs, Cornell University, Ithaca, N. Y.
P. D.: Parke-Davis Co., Detroit, Mich.
N. C. T. C.: Nat. Coll. Type Cultures, Lister Inst., London, Eng.
G. J. H.: Freshly isolated by author.

SELECTION OF TESTS AND OUTLINE OF METHODS

In order to determine which of the tests are the most useful in the classification of the micrococci, a preliminary investigation was made with a small series of cultures and a large number of tests. After summarizing the results of this preliminary survey in which about 40 cultures were used, the following tests were selected for further study as having diagnostic value, *viz.*, ammonia nitrogen assimilation, liquefaction of gelatin, production of ammonia from peptone, production of ammonia from nitrates, nitrate reduction, diastatic action, oxygen requirements, chromogenesis, hemolysis of blood, form and arrangement of cells, nature of cells, fermentation of dextrose, fermentation of lactose, fermentation of sucrose, action on milk, habitat, maximum H-ion concentration production in dextrose broth, nature of agar slant growth, and nature of growth in broth.

Other characters were noted in the preliminary tests but were not considered as of sufficient importance to continue their study for the entire series of cultures. The results as obtained for these supplementary tests are considered later in this paper.

In making a survey of any group of organisms one of the most important points is the adoption of analytical methods which give accurate and constant results. The various methods outlined below were carefully selected after repeated trials with miscellaneous test cultures and such modifications as appear were adopted on account of their special adaptability to the study of this group of organisms.

In all cases the tests were run in triplicate and in many instances the triplicates were repeated many times. The chief object has been to repeat the tests until satisfactory and constant results have been secured.

A routine procedure which has been followed thruout the work was an examination microscopically of all cultures after each test. This is exceedingly important in any pure culture work if any reliance is to be placed on the results as cultures will become contaminated even when handled with the greatest of care. Many of these contaminations can be detected easily from the appearance of the growth and especially is this true when one is familiar with the group under observation, but a surprising number will show foreign organisms in microscopic preparations when the growth appears normal.

All the cultures were carefully examined before the work began to determine their purity and the original preparations were compared at

various intervals with similar preparations made from time to time from the cultures being tested.

In examining the cultures at various intervals for impurities, the Gram stain was essential as a method of eliminating foreign cocci which fail to show in a methylene blue preparation.

The detailed methods as used are listed below.

GRAM STAIN

In staining the organisms by the Gram methods listed below preparations were made on large slides from 24-hour, 2-day, 3-day, 5-day, 7-day, and 30-day agar slant cultures, and duplicate sets prepared from broth cultures. (In the case of those organisms which failed to grow on broth the duplicate preparations were made from agar slant cultures.) Such preparations were stained (in triplicate) with five separate procedures as follows:

Method No. 1.—Stirling method. (Hucker and Conn, 1923.)

Method No. 2.—Ammonium oxalate method. (Hucker, 1921.)

Method No. 3.—As outlined by Buchanan (1911).

Method No. 4.—Stain solution. (Same as Buchanan method.)
Lugol's iodine solution.....1 minute.
Alcohol, until no more color is removed.
Pyronin (1 to 10 alc. aq. sol.).....30 seconds.

Method No. 5.—Stain solution. (Same as Buchanan method.)
Lugol's iodine solution.....1 minute.
Acetone, until no more color was removed.
Pyronin (as above).

The preparations were stained by each of these methods and two series were prepared with the ammonium oxalate method, making a total of six stained series.

The results were recorded as follows:

Gram positive: Retained the violet stain with all methods.

Gram negative: Decolorized in all cases.

Gram variable: Retained the violet stain in one-half of the cases.

Gram variable, generally positive: Decolorized in one or two methods and retained the violet stain by the remaining methods.

Gram variable, generally negative: Retained the violet stain in one or two of the methods and decolorized in remainder of cases.

In those instances where cultures of various ages were used if a discrepancy occurred between the various individual preparations due to the age of the culture, the results recorded were taken from the reaction of the majority of the preparations.

AMINO NITROGEN ASSIMILATION

Cultures were incubated at an optimum temperature for each culture on slants of the following composition in order to determine their ability to utilize amino nitrogen.

Bacto-agar	15.0 grams
Potassium chloride	0.2 gram
Fermentable sugar	10.0 grams
Sodium asparaginate	1.0 gram
Potassium phosphate (dibasic)	1.5 grams
Water (dist.)	1.0 liter
Reaction adjusted to pH 7.0	

One cc. each of 1.6 per cent alcoholic solution of brom cresol purple and cresol red were added as an indicator.

The sodium asparaginate presumably supplied the only available nitrogen as the agar on analysis (washed Difco agar) gave only 0.22 per cent total nitrogen.

The tubes were also thoroly cleaned in "cleaning solution" to eliminate the possibility of introducing further organic material.

The ability of the cultures under observation to utilize the amino nitrogen was determined by the presence or absence of growth which, in turn, was decided by the presence or absence of acid in the medium as indicated by the change in color of the medium due to the action of the indicator added. This method proved satisfactory as only sugars which were available to the strains being tested (usually dextrose) were used and, in many cases where no growth could be observed, large amounts of acid were formed, indicating that growth must have taken place. In no case was growth repeatedly observed without the fermentation of the sugar, providing the sugar was available to the organism in question.

In the case of the organisms which failed to grow, checks were run on normal nutrient media to which the same sugar and indicator were added, and in those cases in which organisms grew (produced acid) repeated inoculations were made on a similar synthetic media. Strains which developed acid, indicating growth, for several generations on

the same medium were classed as types which had the power to utilize amino nitrogen in the form of sodium asparaginate.

AMMONIA NITROGEN ASSIMILATION

The following synthetic medium was used as slants upon which the organisms were incubated at their optimum temperature:

Bacto-agar	15.0 grams
Ammonium phosphate (monobasic)	1.0 gram
Fermentable sugar	10.0 grams
Potassium chloride	0.2 gram
Water (dist.)	1.0 liter

Reaction adjusted to pH 7.0

One cc. each of 1.6 per cent alcoholic solution of brom cresol purple and cresol red were added to each liter of the medium.

Washed Difco agar was used as in the case of the sodium asparaginate agar.

The ability of the organisms to use the ammonia as the only source of nitrogen was determined similarly as in the case of the amino nitrogen, using the presence or absence of acid to denote growth or absence of growth of the strains under observation.

Similar checks were used and, in the case of those organisms which grew, repeated transfers were made on the ammonium phosphate medium to determine if the organisms were utilizing the ammonia nitrogen and not attacking organic material carried over in the inoculum.

LIQUEFACTION OF GELATIN

Two methods were used as a preliminary test for liquefaction of gelatin. In one case a medium was used containing:

Bacto-gelatin	40 grams
Water (dist.)	1.0 liter.

Reaction adjusted to pH 7.0

Duplicate inoculations were carried on the following medium:

Bacto-gelatin	40 grams
Beef extract	3 grams
Peptone	5 grams
Water (dist.)	1.0 liter

Reaction adjusted to pH 7.0

After several trials the latter formula alone was used as it indicated

a tendency to give more uniform results due, no doubt, to the fact that a more characteristic growth developed.

The cultures were incubated at 18° C. for three months[4] and readings were taken every two days.

DIASTATIC ACTION

The ability of the cultures to attack starch was determined by streaking the organisms on the surface of an agar plate which contained a nutrient agar with 1 per cent starch added (Allen, 1918), allowing the plate to incubate for seven days, and then flooding the surface of the medium with Lugol's iodine solution. A clear area around the growth was interpreted as indicating the action of diastase on the starch contained in the medium.

OXYGEN REQUIREMENTS

The oxygen relations of the cultures were noted by observing the amount of growth in deep agar stabs containing a fermentable sugar. There were no obligate aerobes or obligate anaerobes present and this method gave as accurate comparative results as any of the more elaborate procedures. Those cultures which produced the best growths on and near the surface of the slants were classed as facultative anaerobes while those preferring the deep stab were recorded as microaerophilic.

HEMOLYSIS OF BLOOD

The presence or absence of hemolyzing properties in the cocci was determined by adding 1 per cent defibrinated blood to agar just previous to pouring the plates. The organisms tested were incubated on the surface of the medium and the presence of a hemolyzed area around the growth after a period of incubation indicated the presence of hemolytic properties of the organisms under question.

REDUCTION OF NITRATES

The reduction of nitrates was determined by the agar slant method as described by Conn and Breed (1919), and also outlined in the methods published by the Committee on Bacteriological Technic of the Society of American Bacteriologists (1923). Duplicates were run in nitrate broth. Ordinary nutrient agar medium was used to which had been added 0.2 per cent potassium nitrate. At 1-, 2-, 3-, and 4-day intervals, a few drops each of the following reagents were added to the surface of the slant: (a) Solution prepared by dissolving 8

[4]The cotton plugs were sprayed with 5 per cent phenol solution at various intervals to prevent the intrusion of mold spores.

grams of sulphanilic acid in 1 liter of 5N acetic acid, and (b) solution of 5 grams α-naphtylamine in 1 liter of 5N acetic acid. The usual deep red reaction denoted the presence of nitrite, indicating the reduction of the nitrates.

PRODUCTION OF AMMONIA FROM PEPTONE

The ability of the cultures to produce ammonia from organic material in the form of peptone was determined on agar slants by the method as proposed by Hucker and Wall (1922) and Ayers, Rupp and Mudge (1921).

PRODUCTION OF AMMONIA FROM NITRATES

The power of the cocci to produce ammonia from nitrates was tested by inoculating the cultures on agar slants containing the following:

Water (dist.)	1.0 liter
Bacto-agar	15.0 grams
Dextrose	10.0 grams
Potassium nitrate	1.0 gram
Calcium chloride	0.5 gram
Potassium phosphate (dibasic)	0.5 gram
Reaction adjusted to pH 7.0	

The organisms after incubation at an optimum temperature were tested with the phenol-hypochlorite solutions as in the case of ammonia production from peptone. The results were read in three groups, *viz.*, (a) organisms failing to grow, (b) organisms which grew but produced no ammonia, and (c) organisms which grew and produced ammonia.

CHROMOGENESIS

To determine the exact chromogenesis of the various cocci, small portions of a three-weeks-old slant were transferred to the surface of smooth glazed paper and, after drying, compared with the color chart as submitted by the Winslows (1908) and also with the color charts in Ridgeway's color standard. Neither of these methods was entirely satisfactory for several reasons, the principal one being the difficulty of comparing accurately the bacterial growth with the artificial colors as reproduced. Especially is this true in separating the orange and yellow cultures which grade into each other. As a final resort, the cultures were grouped into four groups, *viz.*, white,

yellow, orange, and red, and such a classification served as a basis for the determination of this character.

The definite color as compared with the two color charts, as well as the general grouping of the organisms, was carried out at various ages of growth. Color determinations were also made from gelatin and, in some cases, broth.

FERMENTATION OF SUGARS

The agar slant method (Conn and Hucker, 1920) was used in all cases. It consists in adding the desired sugar (sterilized at 100° C. on three successive days) to the nutrient agar to which is added 1 cc. each of 1.6 per cent alcoholic solution of brom cresol purple and cresol red per liter, and the reaction adjusted to pH 7.0. The medium is inoculated in the same manner as an ordinary agar slant. The change of color denotes the presence of acid or alkali.

In the case of those organisms which were found capable of utilizing ammonium salts as a source of nitrogen, a synthetic, peptone-free medium (Ayers, Rupp, and Johnson, 1919) was used as the basic medium to which was added the test sugar and the indicators. For the cultures which failed to grow under such conditions the ordinary peptone-agar was used.

MAXIMUM H-ION DETERMINATION

The cultures tested for maximum H-ion concentration were grown in the following medium for seven days:

Peptone	5 grams
Meat extract	3 grams
Dextrose	10.0 grams
Water (dist.)	1.0 liter

Reaction adjusted to pH 7.0.

At the completion of the incubation period two 10-cc. samples of the medium were taken and 10 drops of brom thymol blue added to one and a similar amount of cresol red to the other. The colors were then compared with the aid of a comparator, with a set of standards as proposed by Gillespie (1920) prepared by adding various amounts, in drops, of standard acid and alkali solutions to 10 cc. of water.

INDOL PRODUCTION

The following medium was used:

Water (dist.)	1 liter

Peptone	10 grams
Meat extract	5 grams
Sodium chloride	0.5 gram
N/10 sodium hydroxide	7 cc.

To this was added 0.2 gram trypsin and also enough thymol and chloroform to make a layer at top and bottom; the whole digested at 37° C. for two days; diluted 1 to 3 with physiological saline; inoculated, incubated for five days, and tested for indol with the Ehrlich, Salkowski and vanilin tests.

FORM OF CELLS

The presence of regular packets or irregular masses was determined by microscopic examinations of agar slant and broth cultures of various ages.

NATURE OF CELLS

The nature of the cells, whether regular or irregular in shape and size, was determined as above by microscopic examinations at various ages of growth on broth and on agar slants. The results were recorded as (1) regular, indicating that the size and shape of the various units were equal; or (2) irregular, denoting that more or less variation occurred.

ACTION ON MILK

The organisms studied were inoculated into litmus milk and the usual reactions noted.

HABITAT

The habitat was recorded as follows: Cultures from internal human lesions, surface of human bodies, internal animal lesions, surface of animal bodies, udder, milk, water, air, cheese, miscellaneous saprophytes, and unknown.

DATA

Characters applicable to the differentiation of species must evidence a certain amount of constancy when studied over a long series of tests. Furthermore, characters adapted to the differentiation of larger natural groups or genera should, in addition to constancy, show some correlation with other constant characteristics. The presence of this relationship or correlation between characters selected

for the division of genera indicates that the groupings are being made along natural, rather than artificial, lines.

The group of irregular, mass-forming cocci, of which a series of representative cultures has been studied in this investigation, is frequently divided into two genera, *viz.*, *Micrococcus* (saprophytic) and *Staphylococcus* (parasitic). Before such a division should be recognized it should be shown that there is a correlation between two or more characters which serve as a division line in establishing such genera. In order to determine if correlations exist which would warrant the recognition of two separate genera, the results of each test have been compared with the results of all the other tests. The results, together with the general characters of the group, are discussed below.

CHARACTERS OF PRIMARY IMPORTANCE IN THE DIFFERENTIATION OF THE MASS-FORMING COCCI

CHROMOGENESIS

The presence or absence of pigment in coccus cultures has for sometime been used as a diagnostic feature in separating genera, species, or varieties, depending upon the investigator's conception of the importance of this character. It has been generally adopted as it is an easy character to determine and one that has been considered constant by many investigators. Its significance has also been emphasized because of its possible relation to certain other characters of the group. For example, it has been commonly believed that there is a close correlation between pigment production and habitat, Gram staining reactions, etc., which will be discussed in more detail later.

In 1884, Rosenbach, and in 1885, Passet, studying cocci from infected wounds, pointed out that the presence or absence of an orange pigment was of sufficient importance to aid in designating different species of staphylococci. Passet and also Fischer and Levy (1893) believed that the more lightly colored or colorless forms were more generally associated with disease, while Rosenbach was of the opinion that the pigmented types were the most virulent. Noguchi (1911) studied the relationships of virulence to chromogenesis as did Nicolle and Césari (1914), and the results of both of these investigations indicate that the non-chromogenic forms are non-virulent, while Dudgeon and Sargent (1906) reported a number of cases in which

white cocci were isolated from healing wounds and orange strains from infected wounds. The Winslows (1908) also shared the view that the pigmented forms were the most virulent and most workers familiar with the group have agreed more or less with this idea.

The use of chromogenesis in classification was quickly adopted by Flügge (1886), following the work of Rosenbach and Passet, and pigment production was used as a character in separating both genera and species of cocci. Later the Winslows (1906, 1908), Kligler (1913), Winslow, Rothberg, and Parsons (1920), the Committee of the Society of American Bacteriologists (Winslow *et al.*, 1917, 1920), and others have adopted this character and based their systematic arrangements of the mass-forming cocci on the relation of pigment production to habitat, especially in describing genera.

However, opinion as to the importance of chromogenesis has differed and not all workers have agreed that pigment production is of primary and fundamental importance. Neisser and Lipstein (1903) thought that white and chromogenic strains have all other characters in common, and these workers have suggested that all white forms have been derived from pigmented ones due to the action of some external factor which has caused the organism to loose its power of pigment production. They have also pointed out that various factors such as too strong light, too long cultivation in complete darkness, as well as the presence of certain chemicals, may appreciably affect the amount of color production. Longard (1886) also believed that orange and white forms from whatever source were similar, and Dudgeon (1908) thought all staphylococci belonged to one great species regardless of chromogenesis. Neumann (1897), on the other hand, records the appearance of apparently new strains of an entirely different color in various parts of a sealed stab culture which had been allowed to grow for sometime.

Various observations have been reported from time to time concerning the possible relation of the colorless to the colored forms. Rodet and Courmont (1890) stated that they had observed over long periods of time certain white strains which gradually acquired the power to produce a typical orange pigment. Lannelongue and Achard (1890) disagreed with this statement and believed that there were other factors which were definitely correlated with color, such as virulence, size of cells, etc., indicating that white and orange types were different, and questioned the validity of Rodet and Courmont's observations and the purity of their cultures.

Of the 316 representative strains, of mass-forming cocci studied in the present investigation, 110 produced a yellow, 4 a red, and 50 an orange pigment, while 152 failed to show any pigment production at any stage of their growth, when grown at an optimum temperature, reaction, etc. Of the latter, 103 were of the transparent white types, while the remainder gave a chalky opaque growth.

The outstanding fact which is noted at once is the relatively small number of orange and red strains and the large number of white and yellow strains when such wide range of cultures were collected and isolated from various sources. This may be due, in part, to the fact that the orange and red cultures represent selected cultures from stock collections, while a larger number of white and yellow forms were freshly isolated. However, so few red and orange types were found in the isolations that the author believes that the yellow and white forms predominate in nature. Another thing which may affect the number of orange cultures is the difficulty in drawing a boundary line between the orange and yellow cultures, and also the fact that in many laboratories all pigmented mass-forming cocci other than red are called "*aureus*" even in some cases where the pigment is light yellow. In many cases, also, the yellow strains show a distinct buff color at certain stages of their growth which, if only one observation is taken, may cause the particular culture in question to be classed with the orange group whereas its natural pigment may be yellow. The cause of such a development appears to be unknown but may be closely linked with the age and the temperature of growth. To tabulate a culture definitely as orange, the transfer should show a distinct buff to definite orange pigment which usually develops after 24 to 48 hours incubation, the yellow cultures becoming pigmented generally previous to this time.

Color charts have been advocated by several observers interested in the use of chromogenesis as a diagnostic feature in classification and if such charts could be accurately used, it might be possible to separate the yellow-orange group into three sub-groups, *viz.*, (a) those which are constantly and distinctly yellow, (b) a variable group, and (c) a group which develops an orange color. Such an arrangement seems at present impossible, however, as a comparison of a bacterial growth with a color standard is not satisfactory when fine distinctions are to be made. A division of the mass-forming cocci into the large sub-groups of yellow, orange, and red is much to be preferred to a number of smaller groups divided by finer differ-

ences in tint or shade. Most pigmented cultures will vary in shade within certain limits. Thus differences in amount and degree of color are not of as great importance as the presence or absence of color.

As a control on the rough grouping of the cultures according to pigment production on an agar slant, each of the cultures were dried on glazed white paper and compared with both Winslow's color chart for the cocci and Ridgeway's color standard. Such observations were made several times during the course of the work and at various ages of the cultures. In comparing the grouping according to chromogenesis as observed on the agar slant with that as determined by drying on paper, only a few discrepancies were noted. Certain cultures which appeared as orange strains on the surface of agar gave a definite yellow color when dried and in a few instances the reverse was true. However, when the entire series of cultures as studied were divided on the basis of color production and the differentiation determined upon broad lines, the groups so formed appeared relatively constant and subject only to slight variations. Such slight variations may be expected in any character.

A rather close relationship has been claimed by many systematists between chromogenesis and the reaction to the Gram stain. Generally speaking, the saprophytic types (red, yellow, and a few white) are supposed to be Gram negative while the more parasitic forms are regarded as being Gram positive. This holds true in the present work in only a small percentage of the cases. All the cultures were Gram stained with five different methods as described above and Table 2 is arranged to show what relationship, if any, exists between the Gram reaction and the pigment produced. As an examination of the table (pages 34-35) will show, the four red cultures were all variable with a tendency to negative; while the yellow group, commonly associated with the red-pigment-producing types because of their similar habitat, were about equally Gram positive and Gram negative. The orange and white groups, however, were generally positive with a relatively large number of variable strains. In the white group the transparent types were especially constant in being Gram positive, while the "chalky" types tended to be more variable. These results indicate that the red types comprise the only group which tends to be Gram negative, while the remaining cocci are more or less variable with a tendency in the orange and white types to be Gram positive. Further discussion of this point will be found under the general discussion of the Gram stain.

A similar correlation was found to exist between habitat and pigment production. The red types were all from definitely known or probably saprophytic habitats, a condition which agrees with the general conception of this group, while the yellow group appears

TABLE 2.—CORRELATION BETWEEN CHROMOGENESIS

CHROMOGENESIS	NO. OF CULTURES	GRAM STAIN				HABITAT				LIQUEFACTION OF GELATIN		NITRATE REDUCTION		DIASTATIC ACTION		OXYGEN REQUIREMENTS	
		Positive	Var. gen'ly positive	Variable	Var. gen'ly neg'tive	Negative	Misc. parasite	Udder	Milk	Misc. saprophyte	Unknown	Positive	Negative	Reduced	Not reduced	Positive	Negative	Facul'tive anaerobe	Microaerophilic
Red	4	0	0	3	1	0	0	0	0	3	1	2	2	4	0	1	3	4	0
Yellow	110	7	42	24	34	3	35	4	42	17	12	98	12	90	20	13	97	78	32
Orange	50	5	28	1	16	0	37	0	1	3	9	49	1	49	1	3	47	24	16
White	152	27	74	18	29	4	115	3	6	14	14	111	41	129	23	5	147	38	114
Chalky	34	3	20	4	6	1	20	0	2	4	8	19	15	24	10	2	32	20	14
Transparent	103	21	48	10	21	3	84	2	4	8	5	79	24	92	11	3	100	12	91
Miscellaneous	15	3	6	4	2	0	11	1	0	2	1	13	2	13	2	0	15	6	9

about evenly distributed between a parasitic and saprophytic origin. In the orange and white groups parasites are predominant.

Some points of interest were found in comparing the relationship between the reduction of nitrates and chromogenesis. The red cocci, without exception, reduced nitrates. A large majority of the yellow forms appear to attack nitrate, 20 cultures failing to reduce; while the white types, especially the more truly parasitic strains, tend to be non-reducers more frequently than the red and orange groups. The fact that both the white types which are the more parasitic and the red types which represent in general the more saprophytic forms reduce nitrates in nearly all cases makes nitrate reduction a character of little value in characterizing genera.

Only general statements can be made in regard to the fermentation of the sugars—lactose, dextrose, and sucrose. The red and yellow strains tend to be less active than the white and orange groups, as a smaller number of cultures from the former group attacked the test sugars used. In the case of the white group, the "chalky" types are less active than the transparent forms. This may be due to the fact that the former group represents a large number of free-living organisms which in all cases are usually less active when tested against the various fermentable substances. The same relationship is also shown

in the fermentation of glycerin and mannite, substances which are not listed in the table. The chalky white types attack these particular substances in only a few instances.

The relationships of the various groups to the assimilation of amino

AND OTHER CHARACTERS OF THE MICROCOCCI.

Sugar fermentation						Amino nitrogen assimilation		Ammonia nitrogen assimilation		Ammonia production from				Hemolysis of blood		Maximum H-ion concentration in dextrose broth			Action on milk					Nature of cells	
Dextrose		Lactose		Sucrose						Peptone		Nitrate							Acid		Rennet		Peptonization		
Acid	No acid	Acid	No acid	Acid	No acid	Growth	No growth	Growth	No growth	Positive	Negative	Positive	Negative	No growth	Positive	Negative	pH 5.0 to pH 4.0	pH 7.0 to pH 5.0	pH 8.0 to pH 7.0	No growth	Suff'ent to curdle	Not suff't to curdle	No acid	Present	Absent	Present	Absent	Regular	Irregular
2	2	2	2	2	2	0	4	3	1	4	0	0	2	2	0	4	4	0	0	0	1	1	2	0	4	0	4	0	4
94	16	82	28	69	41	71	39	75	35	105	5	17	84	9	8	102	75	1	23	11	63	12	35	15	95	42	68	82	28
49	1	48	2	47	3	13	37	6	44	49	1	2	24	24	3	47	33	14	2	1	40	6	4	1	49	14	36	28	22
140	12	126	26	136	16	40	112	26	126	145	7	5	126	21	29	123	124	10	13	5	98	19	35	6	146	49	103	91	61
27	7	24	10	27	7	14	20	10	24	31	3	2	26	6	4	30	25	3	3	3	15	6	13	2	32	7	27	22	12
99	4	89	14	96	7	18	85	11	92	99	4	3	87	13	23	80	85	7	10	1	70	12	21	3	100	38	65	67	36
14	1	13	2	13	2	8	7	5	10	15	0	0	13	2	2	13	14	0	0	1	13	1	1	1	14	4	11	2	13

and ammonia nitrogen are striking when divided on the basis of chromogenesis. The yellow strains produced an abundant growth when either an amino acid group or ammonium salts were furnished as the only source of nitrogen. In the case of the red group, ammonia nitrogen was generally utilized, while all cultures failed to produce a growth when amino nitrogen was furnished as their only nitrogen source. From Table 2 it can be seen that of 110 yellow cultures studied, 39 failed to utilize amino nitrogen, while of the same cultures 75 produced a growth when nitrogen was furnished in the form of ammonium salts. Among the white types it was noted that a large majority of the transparent strains failed to grow either in the presence of amino or ammonia nitrogen. A similar correlation was noted in the orange group.

The action of the cultures upon milk in relation to chromogenesis has, in many cases, shown correlations, but in the figures given the only apparent differences in the various chromogenic groups is the fact that a large number of the yellow and transparent white types produce varying amounts of peptonization in milk. In some instances peptonization is slight, but in some cases the curd was completely digested. No significant correlations appeared in the study of the other groups.

Many laboratory workers handling large numbers of coccus cultures feel that there is a distinct difference between the white strains which produce a characteristic transparent scanty growth on the surface of agar slants and the strains which grow with an opaque "chalky" appearance. From Table 2 it can be noted that this conception is supported by the present observations in a large number of cases.

The cultures producing a chalky growth tended to react as do the free-living strains, while the general characters of the transparent types are like those of cultures of parasitic origin. This is not an absolute difference, however, and such a division of the white forms would not warrant further grouping by this character other than to estimate roughly the probable source and characteristics of the culture. The length of time which a culture has been grown on artificial medium, together with the composition of the medium, may play an important part in determining the nature of the white growth, and too much reliance should not be placed upon this character until more is known regarding the controlling factors.

In summing up the relationship between chromogenesis and the various characters of this group, it can be readily noted that in only a few instances were correlations found. It appears that the red cocci are the least active from the standpoint of sugar fermentation, gelatin liquefaction, etc., while the transparent white types are the most active of the cultures under observation. A partial correlation was found between habitat and pigment production and in a few cases a relationship appeared to exist between the production of color and Gram staining properties, nitrate reduction, and the ability to utilize various forms of nitrogen.

As to the *nature* of the pigments it is generally agreed from the work of Schrötter (1895), Sullivan (1906), and others that they are lipochromes, or lipoxanthines according to Schrötter.

Neisser (1912) states that Beijerinck believed the micrococcus pigment to be an excretory product and that Zopf found coccus pigments to spot paper and give an acrolein test. To secure further evidence upon this point a representative number of the cultures were grown upon large agar slants and also in Roux flasks, and after the pigment production had reached its maximum, the growth was removed with a spatula and transferred to a watchglass and dried at 37° C. for several days. The dried cultures were then subjected to the action of several solvents with the following results:

Solvent	Number of days extracted†				
	1	2	3	4	10
Alcohol 95 per cent	—	—	—	—	—
Ether	—	—	—	—	±
Chloroform	—	—	—	—	—
Water	—	—	—	—	—
Benzol	—	—	±	±	+

† + denotes dissolving pigment.

In the case of the benzol the extraction was carried on for several weeks and the supernatant fluid only became slightly colored, while the pigment was never entirely removed from the dried culture. In most instances the yellow pigment was removed more quickly than the orange and red pigments. Later it has been noted that Lingelsheim (1900) secured a more efficient extraction if the cultures were ground in acetic acid before dissolving with ether.

When examined with a spectroscope, the red pigment solutions showed a large absorption band in the red and purple portion of the spectrum, while the yellow and orange solutions gave faint bands in the red, yellow, and purple segments of the spectrum.

Repetitions of the above experiments, using heavy saline suspension of the organisms from which to extract the pigment, gave negative results with all of the solvents used.

Of the *factors controlling* the production of pigment little appears to be known. Beck and Schultz (1896) reported the effect of the X-ray and also monochromatic light upon pigment production, while Lubinski (1894) believed that color production was closely linked with certain physiological activites and pointed out that growth under anaerobic conditions intensifies the pigment as well as subjection to an atmosphere of hydrogen and carbon dioxide. Kolle and Otto (1902) thought that temperature must play an important rôle as they observed that cultures subjected to 85° C. lost their power to produce color, while animal passage and too long cultivation on artificial media produced the same effect.

In an extended investigation of the general nature of bacterial pigments Sullivan (1905) pointed out that several factors may be involved. Reaction of media, temperature of growth, and presence of albuminoids appeared to be among the more important, while several minor factors had an influence, depending upon the type and condition of the culture.

Further data were secured by observing the effect of *reaction* of the

medium. It is generally believed that a greater amount of color is produced on an alkaline medium and that the presence of appreciable amounts of acid will retard pigment production. A representative number of the cultures (three yellow and two orange strains) were selected and grown for three generations upon media ranging in acidity from pH 2.0 to pH 10.0. (In the case of the media of the pH 2.0 reaction the agar failed to solidify.) Altho reaction appeared to play an important part in the time of the initial appearance of the pigment, the amount and nature of the color was not affected by the change in H-ion concentration. During the three generations, the color remained about normal varying only within the limits generally observed upon media of the same reaction. While it has been suggested that a number of transfers over a long period of time may show a relation between reaction of media and color production, the present results do not give any foundation for such a belief.

The *temperature* of incubation appears to play an important part in the control of pigment production as well as the temperature at which the culture is normally held as a stock. The orange cultures appeared to form pigment well at 30° to 37° C., while the red types produce their pigment very slowly at this temperature. Room temperature in most cases appears to be the best adapted for color production. Stock cultures held at room temperatures and then transferred will retain their pigment-producing properties much longer than cultures held at ice-chest temperatures and then transferred. Pigmented strains, when carried for several generations under chilled conditions, will loose their color; but it can be regained, if the culture has not been chilled too long, by holding the culture at room temperature followed by several rapid transfers. Transfers of several hundred cultures of micrococci held at both room and ice-chest temperatures have indicated that the temperatures at which the stock cultures are stored plays an important part in the control of color formation in cultures on artificial media.

Rapid transfer of many cultures appeared to affect the intensity of the pigment produced, while in other strains a few rapid transfers would cause the organism practically to loose its color. Of the cultures studied there appeared to be no definite correlation between any one type of pigment and this phenomenon and, as stated above, the temperature as well as length of time of the holding of stock cultures may play a part.

Large amounts of *water of condensation* in nearly all cases appeared

to hinder the production of color. Occasionally, white strains transferred from tubes containing a large amount of water of condensation to relatively dry agar would immediately show a heavy pigment production. Repeated transfers on a medium with abundant water of condensation would cause the culture to loose its pigment-producing power. Konrádi (1904) noted practically similar results, but found that animal passage restored the orange color to the culture which he used.

Various albuminoids and also organic acids (tartaric, acetic, lactic, etc.) were added to the media to note any effect on color production, but the results were uniformly negative. In several instances the cultures grew upon a medium composed of agar, tap water, and dextrose with normal color produced.

From the above observations it seems evident that pigment production by the mass-forming cocci is a very important character for use in classification. However, due to its general lack of correlation with other characters, it does not appear that the group should be divided into genera with differences in chromogenesis as the chief diagnostic feature. On the other hand, it seems that pigment production is sufficiently constant to warrant its use as a character of importance in differentiating species and when so used should be interpreted along broad lines rather than to attempt to make fine distinctions in shades of color.

HABITAT

A natural system of classification must be arranged upon an evolutionary basis and the history of this development can be partially interpreted from the habitat of the organisms. Thus, those cocci which live on and in the tissues of warm-blooded animals must, from their habitat, be considered from the standpoint of historical geology as of comparatively recent origin. Organisms may develop certain characteristics when continuously subjected to a certain environment resulting in a superficial resemblance between cultures from the same habitat tho truly derived from different sources. Such constant growth in the same habitat may bring organisms together into a more or less homogenous group and the general characteristics of these groups are usually correlated with the environment or habitat of the organisms.

For these reasons the habitat of the cocci has generally been adopted and considered as one of the characters essential in classify-

ing the group into a natural system. The mass-forming cocci present some rather difficult problems when considered in relation to their environment. The distribution of this group seems to be primarily on or in the body of warm-blooded animals, and in water, soil, air, milk and various dairy by-products, etc., as less common habitats. In the case of many organisms isolated from these sources it is difficult to determine whether they are true parasites or saprohpytes. This is more especially true in the case of cultures from a saprophytic habitat, for example, the cocci growing in milk and other similar products may be either true saprophytes or parasites living as saprophytes, as it is common to find large numbers of mass-forming cocci in the mammary glands of mammals. Such forms, altho living as true parasites, are usually able to live as saprophytes in milk and milk products. Similar relations may also hold in the case of water and air, as many of the cocci isolated from these sources, altho occurring in such saprophytic habitats, may have originated from parasitic sources and only occur

TABLE 3.—CORRELATION BETWEEN HABITAT AND OTHER

HABITAT	NUMBER OF CULTURES	GRAM					CHROMOGENESIS				LIQUEFACTION OF GELATIN		NITRATE REDUCTION		DIASTATIC ACTION		OXYGEN REQUIREMENTS	
		Positive	Var. gen'ly positive	Variable	Var. gen'ly negative	Negative	White	Yellow	Orange	Red	Positive	Negative	Reduced	Not reduced	Positive	Negative	Facul'tive anaerobe	Microaerophilic
Milk	49	1	23	10	13	2	6	42	1	0	41	8	37	12	4	45	37	12
Water	8	1	4	2	1	0	2	5	1	0	6	2	7	1	1	7	5	3
Miscellaneous saprophyte	29	0	9	9	7	4	14	12	3	3	24	5	21	8	4	25	21	8
Human	57	6	28	5	18	0	34	13	10	0	48	9	53	4	3	54	11	46
Human (Surface)	105	11	55	12	27	0	67	15	22	0	88	17	99	6	4	101	37	68
Animal	7	0	4	2	1	0	5	0	2	0	6	1	7	0	0	7	4	3
Animal (Surface)	18	8	9	0	1	0	16	1	1	0	15	3	17	1	2	16	4	14
Animal (Udder)	7	2	2	1	2	0	3	4	0	0	5	2	7	0	0	7	4	3

under saprophytic conditions temporarily. Later they may assume parasitic or even pathogenic characteristics if the proper conditions are found. It is evident that the definite determination of the natural habitat, especially of the cocci, is very difficult, as the parasites are not so highly specialized as to be unable to adapt themselves to free-living conditions and the saprophytes are so adaptable that under certain conditions they may become parasitic. The group as a whole appears to be transitory between the two extremes with certain sub-

groups which show tendencies either to parasitism or saprophytism.

That habitat has been considered important as a basis for separating the group is evident from the large amount of work completed in which the object has been to obtain a test which would easily differentiate the parasitic and pathogenic types from the less harmful ones. Andrewes and Gordon (1905) believe that this separation could be made on the basis of fermentation of mannite, while Neisser and Wechsburg (1901), Kolle and Otto (1902), Pröscher 1903), Klopstock and Bockenheimer (1904), Van Durme (1903), Veiele (1904), Kutscher and Konrich (1904), Fraenkal and Baumaun (1905), Flügge (1910), Bruck and Hidaka (1911), Geisse (1913), etc., believe that various serological tests are applicable for such a separation.

As early as 1888, Ullmann believed that habitat might serve as a basis for differentiating the micrococci and isolated cultures from air, mouth secretions of healthy persons, etc., and decided that the patho-

CHARACTERS OF THE MICROCOCCI.

SUGAR-FERMENTATION						AMINO NITROGEN ASSIMIL'N		AMMONIA NITROGEN ASSIMIL'N		AMMONIA PRODUCTION FROM		HEMOLYSIS OF BLOOD		MAXIMUM H-ION CONCENTATION IN DEXTROSE BROTH			ACTION ON MILK					NATURE OF CELLS	
Dextrose		Lactose		Sucrose						Peptone	Nitrate						Acid		Rennet	Peptonization			
Acid	No acid	Acid	No acid	Acid	No acid	Growth	No growth	Growth	No growth	Positive	Negative	Positive	Negative	No growth	Positive	Negative	pH 5.0 to pH 4.0	pH 7.0 to pH 5.0	pH 8.0 to 7.0	No growth	Suff'ient to curdle	Not suff't to curdle	No acid	Positive	Negative	Positive	Negative	Regular	Irregular
42	7	40	9	30	19	40	9	6	43	48	1	8	33	8	2	47	38	2	6	3	26	9	14	5	44	17	32	26	23
7	1	6	2	5	3	5	3	4	4	8	0	0	8	0	2	6	5	0	3	0	7	0	1	0	8	0	8	4	4
18	11	15	14	20	9	17	12	19	10	26	3	4	22	3	2	27	11	6	8	4	10	5	14	2	35	7	22	13	16
57	0	54	3	54	3	5	52	0	57	55	2	1	46	10	11	46	44	9	3	1	43	8	6	2	55	18	39	46	11
103	2	97	8	95	10	20	85	14	91	101	4	6	83	16	13	92	87	6	7	5	76	8	21	11	94	40	65	73	32
7	0	6	1	7	0	2	5	2	5	7	0	0	7	0	5	2	6	1	0	0	7	0	0	0	7	5	2	3	4
17	1	17	1	17	1	4	14	0	18	17	1	0	15	3	3	15	18	0	0	0	12	3	3	0	18	4	14	15	3
5	2	4	3	4	3	3	4	5	2	7	0	1	4	2	0	7	3	0	2	2	2	1	4	0	7	0	7	4	3

genic forms were different from the types found under saprophytic conditions. Concornotti (1899), however, decided, upon a repetition of the above work, that the typical air forms were *Staph. aureus* and *albus* and no different from the micrococci found on or in the human body.

The correlation of habitat, as noted in Table 3,[5] with the other

[5]Table 3 gives the habitat of the cultures used in this survey the sources of which were definitely known. A total of 93 strains were from various saprophytic sources and 187 occurred as ecto-, and endo-parasites. Of the 36 remaining cultures not listed in this table because their exact source was not known, the majority were probably from various saprophytic sources.

characteristics of the micrococci appears to be quite striking in some instances. In general, the cultures from parasitic sources tend to be Gram positive, while the saprophytes are, in a greater percentage of the cases, Gram negative. The great variation of the Gram stain as recorded precludes any attempt to draw definite relationships, but from Table 3 this general tendency may be noted. Of the 6 cultures of known origin which were persistently Gram negative, all were from free-living sources. Of the 194 parasites, 125 were Gram positive or varied with such tendencies, and only 49 varied with a tendency to be Gram negative, while 20 appeared so variable as not to be included in either group.

The relationships between habitat and chromogenesis have heretofore been pointed out to be very close, but the results of this survey as recorded in Table 3 are not striking. The parasites are white in a large number of the cases, while the saprophytes tend to the yellow or red type. The latter forms were probably all from various free-living sources, while the yellow, orange, and white forms represent strains from a variety of sources.

On comparison of the general activity of the parasitic and sapro-

TABLE 4.—THE CORRELATION BETWEEN NITRATE REDUCTION AND OXYGEN

	NUMBER OF CULTURES	HABITAT					GRAM				CHROMOGENESIS				LIQUEFACTION OF GELATIN		DIASTATIC ACTION		OXYGEN REQUIREMENT	
		Misc. Parasites	Udder	Milk	Misc. saprophytes	Unknown	Positive	Var. gen'ly positive	Variable	Var. gen'ly negative	Negative	White	Yellow	Orange	Red	Positive	Negative	Positive	Negative	Facul'tive anaerobe	Microaeroph lic
Reduction of Nitrates																					
Non-reducers	44	11	0	12	9	12	7	18	6	9	4	23	20	1	0	27	17	2	42	34	10
Reducers	272	176	7	37	28	24	32	126	40	71	3	129	90	49	4	233	39	20	252	120	152
Oxygen Requirements																					
Facultative anaerobes	154	56	4	37	26	31	18	59	31	43	3	48	78	24	4	131	23	15	139	—	—
Microaerophilic	162	131	3	12	11	5	21	85	15	37	4	114	32	16	0	129	33	7	155	—	—

phytic groups as indicated by liquefaction of gelatin, reduction of nitrates, and fermentation of sugars, it is plainly evident that the parasitic types are more active than those cultures isolated from other sources.

Due to the fact that the micrococci are found in such a wide variety of sources and due to their ability of adaptation to various

environments, the use of habitat alone as a character in separating the general group is precluded. It is true, also, that no other character will definitely correlate with the source of the different types and bear out any conclusions that different natural groups of micrococci can be secured from different habitats.

REDUCTION OF NITRATES

The reduction of nitrates as a test for classifying the cocci gives very clear cut, concise results, and if this test could be applied as a means of classifying the group under observation more definite divisions could be made than in any other way. The methods as described above invariably give clear cut positive or negative results, and such results are, in a large majority of the cases, constant as upon repetition of this test only very few discrepancies were found.

Several repetitions of this test using a nitrate broth in place of the nitrate agar as described above gave results that agreed in practically all cases. However, a study of the correlation between these results and those secured from other tests does not indicate that it is a character closely related to any of the other cultural character-

REQUIREMENTS WITH OTHER CHARACTERS OF THE MICROCOCCI.

SUGAR FERMENTATION						AMINO NITROGEN ASSIMILATION		AMMONIA NITROGEN ASSIMILATION		AMMONIA PRODUCTION FROM				HEMOLYSIS OF BLOOD		MAXIMUM H-ION CONCENTRATION IN DEXTROSE BROTH			ACTION ON MILK						NATURE OF CELLS		REDUCTION OF NITRATES	
Dextrose		Lactose		Sucrose						Peptone		Nitrate							Acid		Rennet		Peptonization				
Acid	No acid	Acid	No acid	Acid	No acid	Growth	No growth	Growth	No growth	Positive	Negative	Positive	Negative	No growth	Positive	Negative	pH 5.0 to pH 4.0	pH 7.0 to pH 5.0	pH 8.0 to pH 7.0	No growth	Suffi'ent to curdle	Not suffi't to curdle	No acid	Positive	Negative	Positive	Negative	Regular	Irregular	Reduced	
27	17	21	23	23	21	28	16	25	19	43	1	5	32	7	6	38	23	1	15	5	12	6	26	7	37	12	32	24	20	—	—
258	14	237	35	231	41	96	176	85	189	260	12	19	204	49	34	238	213	24	23	12	190	32	50	15	257	93	179	177	95	—	—
130	24	109	45	102	52	95	59	92	62	146	8	17	108	29	20	134	103	10	28	13	82	22	50	18	136	56	98	84	70	120	3
155	7	149	13	152	10	29	133	18	144	157	5	7	128	27	21	141	133	15	10	4	120	16	26	4	158	49	113	117	45	152	1

istics of the group. The 272 cocci which have the power to reduce nitrates are probably of several sub-groups, as an examination of Table 4 indicates that in practically every case groups formed on the basis of nitrate reduction can be more or less equally divided when considered in the light of the various other tests. The 44 non-reducers would be expected to be a more or less homogeneous group,

and this upon further examination proved to be the case. This is shown when a further division of the non-reducers is made upon the basis of chromogenesis and liquefaction of gelatin.

An interesting correlation is found in the relation between ammonia production from nitrates and nitrate reduction. The possibility of nitrate reduction, as indicated by the production of nitrites, being covered up by the production of ammonia directly from nitrates or from the nitrites as rapidly as they are formed has been pointed out in another paper (Hucker and Wall, 1922). A striking example of this sort is found in Table 10 in those cases where ammonia was produced from nitrates, while the nitrite test was negative.

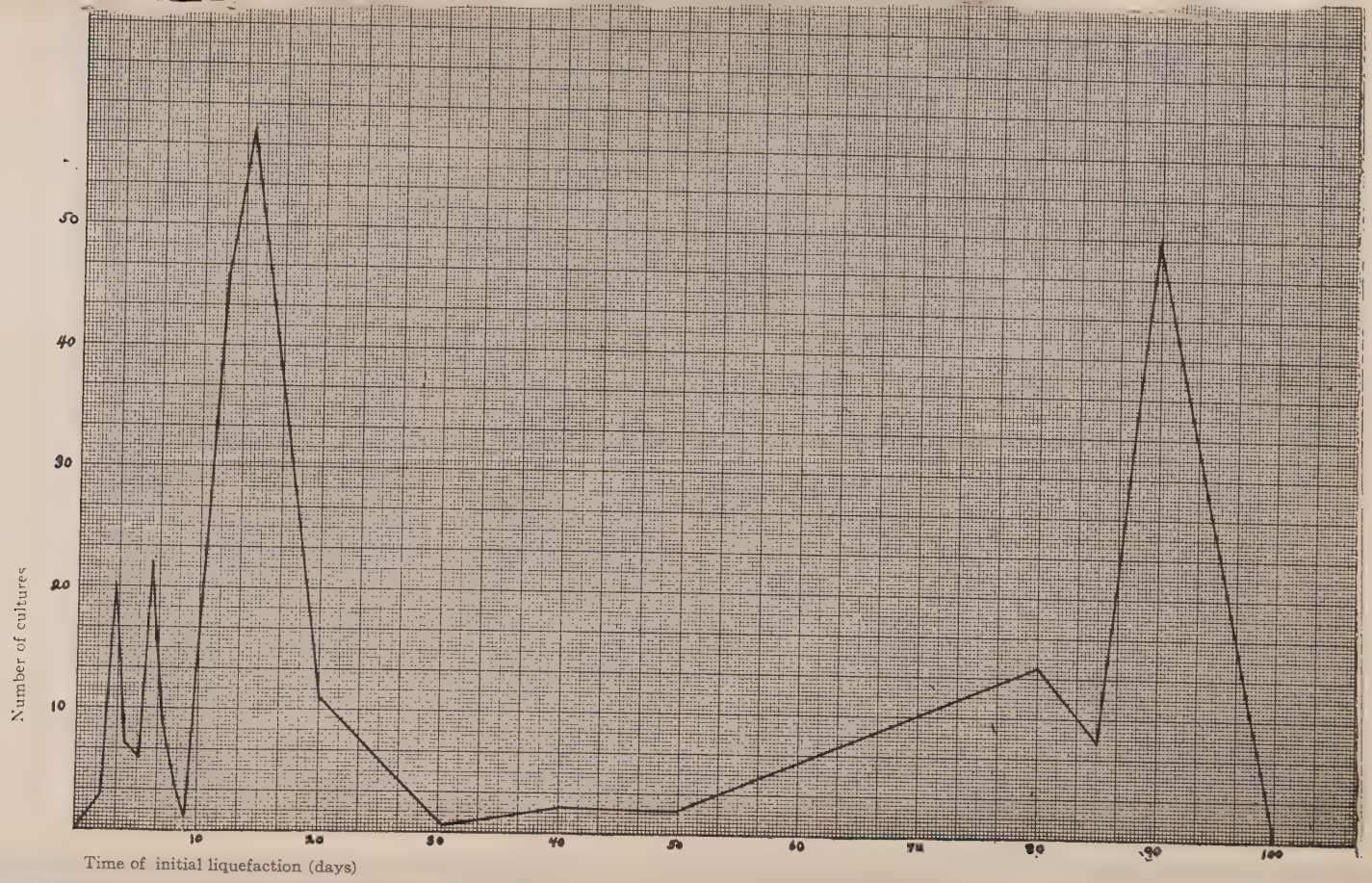
Many other investigators have also considered nitrate reduction as of primary importance in the classification of this group. The Winslows (1908) used this particular test, together with certain others, to differentiate their various species, while Gordon (1905, 1906), in addition to applying nitrate reduction as a diagnostic feature of his group of cocci, believed that nitrate reduction was a character common to all the white micrococci.

Due to its constant and definite reaction, nitrate reduction appears to be especially adapted as a character by which the mass-forming cocci may be further divided into species. Its greatest value, however, is for use in conjunction with certain other tests, particularly chromogenesis and liquefaction of gelatin.

LIQUEFACTION OF GELATIN

Liquefaction of gelatin has been used as a diagnostic character in separating the cocci in many cases and in some instances has been used as the basis for the division of large groups. Chester (1901), Flügge (1886), and Migula (1900) in assembling the various groups of micrococci used this character as a point of departure, while in the hands of other systematists it has proved helpful in classifying the streptococci. Kruse (1896) claimed, however, that liquefaction of gelatin could not be relied upon as cultures carried for some time on artificial media lost their liquefying power. Lannelonge and Achard (1890) thought that the rapidity of the liquefaction was important and pointed out that in many cases *Staphylococcus albus* and *S. aureus* could be separated on this point.

The rate of gelatin liquefaction has also been used by several in separating the so-called *Staphylococcus epidermidis albus* of Welch (1891) from the closely related *S. albus*. Kruse (1896) reported that


Number of cultures
50
40
30
20
10
10
20
30
40
50
60
72
80
90
100
Time of initial liquefaction (days)

repeated cultivations under anaerobic conditions would cause the micrococci practically to loose their power of gelatin liquefaction.

In examining Table 5, it will be seen that a comparatively large number of the cultures under observation possessed the ability to attack gelatin. The Winslows (1908) noted that approximately 128 out of 500 strains (including streptococci) failed to liquefy, while in

TABLE 5.—THE CORRELATION BETWEEN LIQUEFACTION

LIQUEFACTION OF GELATIN	NUMBER OF CULTURES	HABITAT					GRAM			CHROMOGENESIS				DIASTATIC ACTION		OXYGEN REQUIREMENTS	
		Misc. parasite	Udder	Milk	Misc. saprophyte	Unknown	Positive	Var. gen'ly positive	Variable	Var. gen'ly negative	Negative	White	Yellow	Orange	Red	Positive	Negative	Facul'tive anaerobe	Microaerophilic
Liquefied under five days	22	7	2	6	3	4	0	11	6	5	0	3	19	0	0	4	18	20	2
Liquefied between five and ten days.	40	22	0	8	8	2	6	14	10	10	0	21	15	3	1	5	35	20	20
Liquefied between ten and twenty days.	119	75	3	19	9	13	8	57	15	38	1	50	27	41	1	7	112	54	65
Liquefied between twenty and eighty days	23	7	0	6	6	4	2	9	6	4	2	8	12	3	0	2	21	17	6
Liquefied between 80 and 100 days	56	46	0	2	4	4	10	30	5	11	0	29	25	2	0	2	54	20	36
Non-liquefiers	56	30	2	8	7	9	13	24	4	11	4	41	12	1	2	2	54	23	33

the present survey only 56 out of 316 failed to attack gelatin. The Winslows, however, allowed the gelatin cultures to incubate only 30 days, while in the present work a period of 100 days was used.

In examining Chart 1, it will be noted that the length of time of incubation used in determining liquefaction is an important factor in studying staphylococci. Incubating for 30 days only will, in the case of the group under study, give positive results in only about 60 per cent of the total cases which may liquefy in a longer period. From Chart 1 it is evident that at least five groups may be separated on the basis of the appearance of initial liquefaction, *viz.*, (a) those that liquefy before 5 days, (b) those that liquefy between 5 and 10 days, (c) those that liquefy between 10 and 20 days, (d) those that liquefy between 20 and 80 days, and (e) those that liquefy between 85 and 100 days. A far greater number of cultures, however, tend to liquefy before 30 days, but several may give decided reactions as late as the 100th day.

Many workers have used the *type* of gelatin liquefaction, whether craterform, saccate, etc., as a basis for differentiating certain groups

of organisms. Such an application of this test to the mass-forming cocci is not warranted from the results of this investigation. Altho certain strains appeared more or less constant in the type of liquefaction produced, a large number varied considerably. Amount of inoculum, temperature of incubation, composition of media, and any factor affecting the rapidity of the growth appeared, in a majority

OF GELATIN AND OTHER CHARACTERS OF MICROCOCCI.

Sugar fermentation						Reduction of nitrates		Amino nitrogen assimilation		Ammonia nitrogen assimilation		Ammonia production from				Hemolysis of blood		Maximum H-ion concentration			Action on milk				Nature of cells	
Dextrose		Lactose		Sucrose								Peptone		Nitrate							Acid		Rennet		Peptonization		
Acid	No acid	Acid	No acid	Acid	No acid	Reduced	Not reduced	Growth	No growth	Growth	No growth	Positive	Negative	Positive	Negative	No growth	Positive	Negative	pH 5.0 to pH 4.0	pH 7.0 to pH 5.0	pH 8.0 to pH 7.0	No growth	Suffi'ent to curdle	Not suffi't to curdle	No acid	Positive	Negative	Positive	Negative	Regular	Irregular
20	2	16	6	12	10	20	2	19	3	19	3	20	2	3	19	0	2	20	11	2	4	5	13	1	8	6	16	12	10	17	5
34	6	35	5	31	9	37	3	15	25	17	23	40	0	4	31	5	7	33	32	2	4	2	30	3	7	1	39	20	20	27	13
108	11	101	18	95	24	106	13	44	75	37	82	115	4	7	93	19	16	103	90	8	13	8	84	8	27	11	108	47	72	79	40
19	4	18	5	17	6	17	6	13	10	11	12	22	1	4	12	7	3	20	14	1	7	1	13	4	6	0	23	4	19	11	12
56	0	49	7	54	2	53	3	16	40	10	46	53	3	3	41	12	9	47	45	6	4	1	42	8	6	3	53	14	42	39	17
48	8	39	17	45	11	39	17	19	37	17	39	52	4	3	41	12	3	53	48	6	2	0	20	14	22	0	56	8	48	29	27

of the cases, to have its effect upon the type of growth produced.

The appearance of the initial liquefaction seems to be independent of the amount of liquefaction. In many cases the strains showing a decided liquefaction before five days will only produce a slight liquefaction, and even if incubated for long lengths of time will fail to produce greater reactions. On the other hand, many of the cultures will give abundant growth but no liquefaction for 80 days and within 24 hours will entirely liquefy the tube. This is not true in all the cases of slow liquefaction, however, as many of the cultures at the end of long periods of incubation on gelatin produce very slight traces of liquefaction. In the case of those types which appear to lay dormant for sometime and then suddenly attack the medium and rapidly liquefy it, it may be true that during their period of long growth certain constituents of the gelatin which are more available to the organisms are used and that when these ingredients are all utilized, another part of the gelatin is immediately attacked. As a result of this second attack the gelatin is liquefied, or rather the cells autolyze and liberate endoenzymes which attack the gelatin. This apparent

delayed liquefaction appears constant as all of the observations were checked several times and the time of the initial appearance of the liquefaction varied only a few days in most cases. It is evident that to test the liquefying power of the mass-forming cocci the cultures must be incubated at least 100 days.

The organisms possessing the ability to liquefy gelatin according to Table 5 have no other characters in common. However, the non-liquefiers, when further divided upon the basis of chromogenesis, appear to form a definite and more or less constant group. Failure to attack gelatin appears to be a fundamental character and, due also to

TABLE 6.—THE CORRELATION BETWEEN THE ACTION

ACTION ON MILK	NUMBER OF CULTURES	HABITAT					GRAM				CHROMOGENESIS				DIASTATIC ACTION		LIQUEFACTION OF GELATIN		OXYGEN REQUIREMENT	
		Misc. parasite	Udder	Milk	Misc. saprophyte	Unknown	Positive	Var. gen'ly positive	Variable	Var. gen'ly negative	Negative	White	Yellow	Orange	Red	Positive	Negative	Positive	Negative	Facul'tive anaerobe	Microaerophilic
Acid																					
Sufficient to curdle	202	138	2	26	17	19	19	103	28	52	0	98	63	40	1	13	189	182	20	82	120
Not sufficient to curdle	38	19	1	9	5	4	9	17	3	8	1	19	12	6	1	1	37	24	14	22	16
No acid	76	30	4	14	15	13	11	24	15	20	6	35	35	4	2	8	68	54	22	50	26
Rennet																					
Positive	22	13	0	5	2	2	0	9	6	7	0	6	15	1	0	3	19	22	0	18	4
Negative	294	174	7	44	35	34	39	135	40	73	7	146	95	49	4	19	275	238	56	136	158
Peptonization																					
Slight	73	51	0	9	2	11	6	34	13	20	0	40	20	13	0	1	72	66	7	32	41
Abundant	32	16	0	8	5	3	2	16	7	7	0	9	22	1	0	7	25	31	1	24	8
Negative	211	120	7	32	30	22	31	94	26	53	7	103	68	36	4	14	197	163	48	98	113

its constancy, it appears to furnish a feature which can be used in conjunction with chromogenesis to designate smaller groups or possibly species The liquefiers, however, appear to be made up of a very heterogenous group of cultures which precludes the use of this character alone as a basis of separation of groups.

ACTION ON MILK

Investigators have differed in many instances as to the diagnostic value of milk in separating the cocci into their various natural groups. A few workers (Winslows, 1908) with this group have regarded milk as unsatisfactory as it affords only a limited number of tests when used as a medium, *viz.*, change of reaction, coagulation, and decolor-

ization of litmus. However, other observers have reported proteolytic (Gorini, 1910) strains and, in one case, a gas-producing culture (Adametz, 1895) is also described. A French investigator (Marbias, 1919) has made an extensive study of milk reactions in the mass-forming cocci and decided that it serves as an excellent medium with which to differentiate the various groups.

That the mass-forming cocci in many cases have the power to attack milk and produce varying amounts of proteolytic action has been shown by Freudenreich and Thöni (1903), Orla-Jensen (1904), Gorini (1910), etc. These organisms have been isolated from the

UPON MILK AND OTHER CHARACTERS OF THE MICROCOCCI.

Sugar fermentation						Amino nitrogen assimilation		Ammonia nitrogen assimilation		Reduction of nitrates		Ammonia production from				Hemolysis of blood		Maximum H-ion concentration				Nature of cells	
Dextrose		Lactose		Sucrose								Peptone		Nitrate									
Acid	No acid	Acid	No acid	Acid	No acid	Growth	No gorwth	Growth	No growth	Reduced	Not reduced	Positive	Negative	Positive	Negative	No growth	Positive	Negative	pH 5.0 to pH 4.0	pH 7.0 to pH 5.0	pH 8.0 to pH 7.0	No growth	Regular	Irregular
197	5	192	10	174	28	70	132	58	144	190	12	197	5	18	172	12	31	171	161	19	14	8	147	55
35	3	35	3	31	7	16	22	13	25	32	6	35	3	2	36	0	1	37	31	1	5	1	17	21
53	23	31	45	49	27	38	38	39	37	50	26	71	5	4	64	8	8	68	44	5	19	8	37	39
20	2	12	10	13	9	15	7	14	8	7	15	19	3	0	22	0	3	19	14	0	3	5	15	7
265	29	246	48	241	53	109	185	96	198	257	37	284	10	24	214	56	37	257	222	25	35	12	186	108
73	0	70	3	60	13	26	47	21	52	69	4	72	1	4	60	9	9	64	58	8	5	2	46	27
28	4	20	12	22	10	23	9	32	0	24	8	28	4	4	25	3	4	28	20	3	4	5	26	6
184	27	168	43	172	39	75	136	65	146	179	32	203	8	16	151	44	27	184	158	14	29	10	129	82

udder, cheese, milk, etc., and have been considered by many workers in this field to play an important part in the changes occurring in various dairy products.

In the present survey (Table 6) a large number of peptonizing cocci were found, as 105 of the 316 cultures studied attacked casein and 32 of them were very active and rapidly liquefied the curd. The peptonizing strains appear to have no other characters in common, but it can be noted also in Table 6 that the non-pigment-producing cultures have a greater tendency to produce this action in milk than do the pigmented forms.

Only a small number (22) of the cultures produced a rennet curd and this character appears to show no promise as a diagnostic feature

when used alone in describing groups of cocci. Petruschky (1890), Fermi (1890), and Dyar (1895), however, have described alkaline-coagulating types of staphylococci, and this property may be characteristic of certain species.

A study of the activities of the organisms from the standpoint of production of acid in milk has, in many instances, produced some interesting results, the most striking of which is the fact that many of the cocci of this group may produce a smooth acid curd in milk very similar to that of *Streptococcus lactis* and in which the litmus is reduced with the red ring appearing at the top. This character in some cases is accepted as a rough test for the lactic organism. The action in such cases appears to be quite rapid and the smooth curd is formed upon 24 hours incubation. Such acid curd production with the reduction of litmus by the cocci indicates that other cultural

TABLE 7.—CORRELATION BETWEEN THE NITROGEN

NITROGEN	NUMBER OF CULTURES	HABITAT					GRAM				CHROMOGENESIS				DIASTATIC ACTION		LIQUEFACTION OF GELATIN		OXYGEN REQUIREMENTS	
		Misc. parasite	Udder	Milk	Misc. saprophyte	Unknown	Positive	Var. gen'ly positive	Variable	Var. gen'ly negative	Negative	White	Yellow	Orange	Red	Positive	Negative	Positive	Negative	Facul'tive anaerobe	Microaerophilic
Amino nitrogen																					
Utilized	124	31	3	40	22	28	14	52	23	32	3	40	71	13	0	13	111	105	19	95	29
Not utilized	192	156	4	9	15	8	25	92	23	48	4	112	39	37	4	9	183	155	37	59	133
Ammonia nitrogen																					
Utilized	110	14	5	43	23	25	9	51	25	35	4	26	75	6	3	13	97	93	17	92	18
Not utilized	206	173	2	6	14	11	30	100	21	52	3	126	35	44	1	9	197	167	39	62	144

characters should be used in conjunction with the typical curd and reduction of litmus to characterize the common lactic organism generally found in milk.

In general, the parasitic cocci tend to produce acid in milk, but in most instances the amount produced is not sufficient for curdling. This non-curdling group was generally Gram positive or varied with a tendency to be positive, and a large majority were facultative anaerobes. The curdling cocci, 202 in number, appeared to be the most active sugar fermenters, but they were widely variable in their habitat and reaction to the Gram stain.

The cultures which were inert in milk, on the other hand, differed

from the curdling types in being very inactive in relation to other tests, especially in the fermentation of sugars and reduction of nitrates. About 34 per cent of this group fail to reduce nitrates, while a relatively small number produced acid from sugars. In studying the acid production from sugar, one interesting point can be noted in Table 6. Of 76 cultures which failed to produce acid in milk, 31 produced acid in agar slants when lactose was furnished as a source of carbon. This apparent discrepancy was found on repeating the test several times and no explanation can be offered other than a possible simultaneous production of acid and alkali in the milk, whereas in the agar slant the peptone was not as available as the casein and no neutralizing by-products were produced.

Under the same conditions of growth the action of the mass-forming cocci upon milk appears to be comparatively constant, the widest

REQUIREMENTS AND OTHER CHARACTERS OF THE MICROCOCCI.

SUGAR FERMENTATION						REDUCTION OF NITRATES		AMMONIA PRODUCTION FROM				HEMOLYSIS OF BLOOD		MAXIMUM H-ION CONCENTRATION IN DEXTROSE BROTH			ACTION ON MILK						NATURE OF CELLS	
Dextrose		Lactose		Sucrose				Peptone		Nitrate							Acid		Rennet		Peptonization			
Acid	No acid	Acid	No acid	Acid	No acid	Reduced	Not reduced	Positive	Negative	Positive	Negative	No growth	Positive	Negative	pH 5.0 to pH 4.0	pH 7.0 to pH 5.0	pH 8.0 to pH 7.0	No growth	Sufficient to Curdle	Not sufficient to Curdle	No acid	Positive	Negative	Positive	Negative	Regular	Irregular
116	8	93	31	80	44	96	28	116	8	14	93	17	9	115	83	8	22	11	70	16	38	15	109	49	75	77	47
169	13	165	27	174	18	176	16	187	5	10	143	39	31	161	153	17	16	6	132	22	38	7	185	56	136	124	68
89	21	77	33	65	45	96	25	104	6	15	81	14	6	104	66	7	24	13	58	13	39	14	96	45	65	61	49
196	10	181	25	189	17	189	17	199	7	9	155	42	34	172	170	18	14	4	144	25	37	8	198	60	146	140	66

variation occurring in relation to the amount of acid produced. Action on milk due to its relative constancy may be found useful in separating species if the results are properly interpreted.

NITROGEN REQUIREMENTS

As nitrogen is one of the essential and important requirements for bacterial growth, it is evident that organisms with such a wide range of habitat as is found among the mass-forming cocci must have the power to utilize both organic and inorganic forms of nitrogen. The saprophytic mass-forming cocci which are generally found in places where inorganic ammonium salts are also present no doubt have

developed the power of utilizing such salts as their only source of nitrogen. In order to secure evidence on this particular point, the cultures of this series were tested for their ability to grow on media containing ammonium phosphate and sodium asparaginate as the only sources of nitrogen. See page 24 for a detailed statement of the composition of the media.

Table 7 gives a general outline of the results. The most striking point which may be noted on studying the table is the fact that the mass-forming cocci from parasitic sources failed to grow, while the cultures from saprophytic sources grew on a medium containing ammonium phosphate as the only source of nitrogen. Only 14 of the 187 parasites utilized nitrogen when furnished in the form of ammonium salts, and these 14 strains which grew were all ectoparasitic and were isolated from the surface of mammalian bodies.

CHARACTERS OF SECONDARY IMPORTANCE IN DIFFERENTIATING THE MASS-FORMING COCCI

The following characters show so little correlation with other characteristics of the mass-forming cocci that they are not satisfactory

TABLE 8.—THE CORRELATION BETWEEN THE GRAM

GRAM STAIN	NUMBER OF CULTURES	HABITAT					CHROMOGENESIS				LIQUEFACTION OF GELATIN		NITRATE REDUCTION		DIASTATIC ACTION		OXYGEN REQUIREMENT	
		Misc. parasite	Udder	Milk	Misc. saprophyte	Unknown	White	Yellow	Orange	Red	Positive	Negative	Reduced	Not reduced	Positive	Negative	Facul'tive anaerobe	Microaerophilic
Gram positive	39	25	2	1	1	10	27	7	5	0	26	13	32	7	3	36	18	21
Gram variable, generally positive	144	96	2	23	13	10	74	42	28	0	120	24	126	18	8	136	59	85
Gram variable	46	19	1	10	11	5	18	24	1	3	42	4	40	6	8	38	31	15
Gram variable, generally negative	80	47	2	13	8	10	29	34	16	1	69	11	71	9	3	77	43	37
Gram negative	7	0	0	2	4	1	4	3	0	0	3	4	3	4	0	7	3	4

for use as characters of primary importance in classifying the group. In some cases, however, these characters show a certain amount of constancy and for this reason may be useful in further characterizing species.

GRAM STAIN

In previous studies of the systematic relationships of cocci the Gram stain has been found an important means of dividing the group It was probably first used in this connection by Heydenreich (1889) In nearly all cases, however, where this particular test has been used for dividing the cocci, only one method of staining has been tried, a fact which has insured more or less constant results. The reactions of the various groups of cocci to the Gram stain have been generally considered to be definite and constant, and in giving such reactions this character has served as a very suitable diagnostic feature in separating so-called natural groups. The discrepancies which occur in using the Gram stain for separating these groups are readily found when more than one procedure is used and comparisons are made between the results of the various methods of staining. From the variable results (Table 8) in which five procedures were used, it can be readily seen that the use of a single method may lead to erroneous conclusions. The reactions of the cocci to the Gram stain appear to be very variable when tested by a variety of methods. Only a few of the cultures studied were found to be constant in their reaction. Of the 316 cultures under observation only 46 gave constant results

STAIN AND OTHER CHARACTERS OF THE MICROCOCCI.

SUGAR FERMENTATION						AMINO NITROGEN ASSIMILATION		AMMONIA NITROGEN ASSIMILATION		AMMONIA PRODUCTION FROM				HEMOLYSIS OF BLOOD		MAXIMUM H-ION CONCENTRATION IN DEXTROSE BROTH			ACTION ON MILK						NATURE OF CELLS	
Dextrose		Lactose		Sucrose						Peptone		Nitrate							Acid		Rennet		Peptonization			
Acid	No acid	Acid	No acid	Acid	No acid	Growth	No growth	Growth	No growth	Positive	Negative	Positive	Negative	No growth	Positive	Negative	pH 5.0 to pH 4.0	pH 7.0 to pH 5.0	pH 8.0 to pH 7.0	No growth	Sufficient to curdle	Not sufficient to curdle	No acid	Positive	Negative	Positive	Negative	Regular	Irregular
138	1	29	10	34	5	14	25	19	30	37	2	4	22	13	4	35	33	2	4	0	19	9	11	0	39	8	31	26	13
135	9	125	19	121	23	52	92	52	92	139	5	9	107	28	18	126	116	13	11	4	103	17	24	9	135	50	94	101	43
40	6	36	10	33	13	23	23	25	21	43	3	6	36	4	6	40	26	3	12	5	28	3	15	6	40	20	26	28	18
69	11	65	15	50	30	32	48	28	52	77	3	4	65	11	12	68	58	6	9	7	52	8	20	7	73	27	53	45	35
3	4	3	4	6	1	3	4	4	3	7	0	1	6	0	0	7	3	1	2	1	0	1	6	0	7	0	7	1	6

when stained by the five methods mentioned above, and only 7 of these have remained constantly Gram negative to all the procedures.

Another factor which may lead to erroneous conclusions in using the Gram stain is the age of the culture at the time of staining.

As stated above, the cultures were all stained at various ages in their growth and, as recorded in Table 8, the average reaction to the Gram stain of the various ages thus examined was recorded. In general, however, from the standpoint of the age of the culture, it was noted that the organisms lost their ability to retain the violet stain as they became older, and only a few of the strains studied were found to be constantly Gram positive at the end of 30 days. The appearance of the cultures at the end of the 30-day period was very similar, as practically all, at this stage presented an uneven stained background, probably similar to "symplasm" described by Löhnis (1921), covered with the characteristic deeply stained granules. During the 12- to 36-hour stage, however, the cultures stained distinctly with each individual unit giving a clear cut and definite picture, indicating that this particular period is preferable for the best results with the Gram

TABLE 9.—CORRELATION BETWEEN MAXIMUM H-ION CONCENTRATION IN DEXTROSE BROTH AND

	NUMBER OF CULTURES	HABITAT					GRAM				CHROMOGENESIS				DIASTATIC ACTION		LIQUEFACTION OF GELATIN		OXYGEN REQUIREMENTS	
		Misc. parasite	Udder	Milk	Misc. saprophytes	Unknown	Positive	Var. gen'ly positive	Variable	Var. gen'ly negative	Negative	White	Yellow	Orange	Red	Positive	Negative	Positive	Negative	Facul'tive anaerobe	Microaerophilic
Maximum H-ion concentration in dextrose broth																					
pH 5.0 to pH 4.0	236	155	3	38	16	24	33	116	26	58	3	124	75	33	4	14	222	188	48	103	133
pH 7.0 to pH 5.0	25	16	0	2	6	1	2	13	3	6	1	10	1	14	0	2	23	19	6	10	15
pH 8.0 to pH 7.0	38	10	2	6	11	9	4	11	12	9	2	13	23	2	0	4	34	36	2	28	10
No growth	17	6	2	3	4	2	0	4	5	7	1	5	11	1	0	2	15	17	0	13	4
Nature of Cells																					
Regular	201	137	3	26	17	18	26	101	28	45	1	91	82	28	0	14	187	172	29	84	117
Irregular	115	50	4	23	2	38	13	43	18	35	6	61	28	22	4	8	107	88	27	70	45

stain. No definite correlation between the Gram reaction and other characters could be found when the results of the staining were considered as positive, negative, and variable, and this lack of relationship shows that the Gram reaction is not adapted for use in separating the mass-forming cocci into large groups of species. Certain parasitic strains, however, may show sufficient constancy in their reaction to this test to allow its use for their identification, but in no case should reliance be placed upon the results as obtained with a single staining and at least three preparations should be stained before making a

decision as to the probable reaction of the culture under question

Exceptions to this marked variation in staining properties are generally found in Gram stained material from various lesions and in parasitic organisms freshly isolated. In most of these cases where *Staphylococcus albus*, *S. aureus*, and certain closely related species are found, the organisms give a more constant Gram positive reaction. This is not the case when cultures carried for some time on artificial media or strains from saprophytic sources are examined.

MAXIMUM H-ION CONCENTRATION IN DEXTROSE BROTH

The maximum H-ion concentration produced in sugar broths has been found useful in the hands of various workers, particularly for separating the free-living and parasitic streptococci, and has furnished evidence in many cases of differences between these two sub-

NATURE OF CELLS WITH OTHER CHARACTERS OF THE MICROCOCCI.

SUGAR FERMENTATION						REDUCTION OF NITRATES		AMINO NITROGEN ASSIMILATION		AMMONIA NITROGEN ASSIMILATION		HEMOLYSIS OF BLOOD	AMMONIA PRODUCTION FROM				ACTION ON MILK				NATURE OF CELLS		MAXIMUM H-ION CONCENTRATION IN		
Dextrose		Lactose		Sucrose									Peptone		Nitrate		Acid		Rennet	Peptonization					
Acid	No acid	Acid	No acid	Acid	No acid	Reduced	Not reduced	Growth	No growth	Growth	No growth	Positive	Negative	Positive	Negative	Positive	Negative	No growth	Suffi'ent to curdle	Not suffi't to curdle	No acid	Positive	Negative	Positive	Negative	Regular	Irregular	pH 5.0 to pH 4.0	pH 7.0 to pH 5.0	
230	6	208	28	206	30	213	23	83	153	66	170	33	203	228	8	18	168	50	161	31	44	12	224	78	158	158	78	—	—	
22	3	20	5	23	2	24	1	8	17	7	18	3	22	23	2	3	19	3	19	1	5	0	25	11	14	15	10	—	—	
20	18	18	20	17	21	23	15	22	16	24	14	4	34	36	2	2	33	3	14	5	19	3	35	9	29	17	21	—	—	
13	4	12	5	8	9	12	5	11	6	13	4	0	17	16	1	1	16	0	8	1	8	5	12	7	10	11	6	—	—	
192	9	178	23	162	39	177	24	77	124	61	140	25	176	192	9	19	161	21	147	17	37	15	186	72	129	—	—	158	15	1
93	22	80	35	92	23	95	20	47	68	49	66	15	100	111	4	5	75	35	39	55	21	7	108	33	82	—	—	78	10	

groups which, tho morphologically similar, have, in many cases, widely different physiological requirements.

Ayers, Johnston, and Davis (1918) have pointed out that pathogenic strains of streptococci gave final H-ion concentrations varying from pH 5.4 to 5.6, while the non-pathogenic cultures of the same series produced a H-ion concentration of pH 4.5 to 4.9. Avery and Cullen (1919) found that streptococci from bovine sources generally gave higher H-ion concentrations than similar strains from human sources, while Jones (1920) decided that the final H-ion concentration was influenced by such factors as composition and initial reaction of media, amount of inoculum, etc.

The results as obtained with the mass-forming cocci, as indicated in Table 9, do not show any striking correlations. The parasites, generally speaking, appear to be more active than the saprophytes, as a larger number of parasites produce a maximum H-ion concentration, pH 5.0 or over, while cultures from saprophytic sources were generally found to produce the lower H-ion concentrations. The production of varying amounts of H-ion concentration does appear to be a character applicable for use in classifying the mass-forming cocci, but rather serves as an indication of the relative activity of the various forms appearing in this group. The results do not appear to be constant as all of the factors controlling the growth of the organisms play an important part in affecting the variation of the final H-ion concentration.

The mass-forming cocci, altho limited in their range of H-ion pro-

TABLE 10.—THE CORRELATION BETWEEN AMMONIA PRODUCTION

AMMONIA	NUMBER OF CULTURES	HABITAT					GRAM				CHROMOGENESIS				DIASTATIC ACTION		LIQUEFACTION OF GELATIN	
		Misc. parasite	Udder	Milk	Misc. saprophytes	Unknown	Positive	Var. gen'ly positive	Variable	Var. gen'ly negative	Negative	White	Yellow	Orange	Red	Positive	Negative	Positive	Negative
Peptone																			
Produced ammonia	303	180	7	48	34	34	37	139	43	77	7	145	105	49	4	21	282	251	52
No ammonia produced	13	7	0	1	3	2	2	5	3	3	0	7	5	1	0	1	12	9	4
Nitrate																			
Produced no ammonia	236	151	4	33	30	18	22	107	36	65	6	126	84	24	2	16	220	195	41
Ammonia produced	24	7	1	8	4	4	4	9	6	4	1	5	17	2	0	2	22	21	3
No growth	56	29	2	8	3	14	13	28	4	11	0	21	9	24	2	4	52	44	12

duction, will grow in media with a H-ion concentration ranging from pH 10.0 upward. Hall and Fraser (1921) also found micrococci growing in media with a reaction of pH 2.6 to pH 10.0.

AMMONIA PRODUCTION

Ammonia production has usually served as an index to the relative activities of various organisms in relation to certain compounds. The production of ammonia may follow two distinct courses, due primarily to the nature of the nitrogenous substances furnished the organism. This may be either organic or inorganic. In supplying

organisms with organic sources of nitrogen in the form of peptone the production of ammonia in any quantity may possibly indicate that the peptone has been utilized and ammonia produced as one of the chief by-products. In other cases, however, ammonia may be produced from nitrates. Therefore, to state that the organism is an ammonifier without stating the source of nitrogen may lead to erroneous conclusions. The two phenomena, namely, ammonia production from organic and inorganic sources, represent quite different fundamental physiological activities of any group. Table 10 indicates that practically all of the cultures produced more or less ammonia from peptone. A similar observation was recorded by Lübbert (1886), whereas in those cases where nitrate alone was furnished as the source of nitrogen comparatively few produced appreciable amounts of ammonia. In other words, the mass-forming cocci are ammonifiers

AND OTHER CHARACTERS OF THE MICROCOCCI.

Sugar fermentation						Reduction of nitrates		Amino nitrogen assimilation		Ammonia nitrogen assimilation		Hemolysis of blood		Maximum H-ion concentration in dextrose broth			Action on milk				Nature of cells		Oxygen requirements	
Dextrose		Lactose		Sucrose													Acid		Rennet	Peptonization				
Acid	No acid	Acid	No acid	Acid	No acid	Reduced	Not reduced	Growth	No growth	Growth	No growth	Positive	Negative	pH 5.0 to pH 4.0	pH 7.0 to pH 5.0	pH 8.0 to pH 7.0	No growth	Sufficient to curdle	Not sufficient to curdle	No acid	Positive	Negative	Positive	Negative	Regular	Irregular	Facultative anaerobe	Microaerophilic
273	30	247	56	243	60	271	43	116	187	104	199	40	263	228	23	36	16	197	35	71	19	284	100	203	192	111	146	157
12	1	11	2	11	2	14	1	8	5	6	7	0	13	8	2	2	1	5	3	5	3	10	5	8	9	4	8	5
																												1
210	26	200	36	189	47	204	32	93	143	81	155	38	198	168	19	33	16	182	0	64	22	204	85	151	161	75	108	128
23	1	13	11	14	10	19	5	14	10	15	9	1	23	18	3	2	1	18	2	4	0	24	38	16	19	5	17	7
52	4	45	11	51	5	49	7	17	39	14	42	1	55	50	3	3	0	12	36	8	0	56	12	44	21	35	29	27

from the standpoint of organic nitrogen, but do not have the ability to reduce nitrates to ammonia. This, however, does not necessarily signify that the nitrates are not attacked as in a large number of cases the nitrates are reduced to nitrites as described in another section of this paper. The 13 cultures which failed to produce ammonia from peptone likewise failed to show any other common characters. Their activities in attacking other substances such as sugar, gelatin, starch, etc., seemed to be similar to those of the organic ammonifiers. From such observations it may be assumed that the ability of the cocci to produce ammonia does not signify in any way the relative activity of the organism.

The results of the tests to determine the production of ammonia from nitrates are very similar. In this case, however, a large number of the organisms, 56 in all, failed to grow on a synthetic medium where nitrates were the only source of nitrogen. It is interesting to note, in this connection, that 17 of these 56 organisms which failed to grow on nitrates grew on media in which sodium asparaginate was the only nitrogen source, and 14 grew when ammonium phosphate was furnished as the source of nitrogen. In the case of the 24 organisms producing ammonia from nitrate no other common characteristics were found.

In light of the above results, ammonia production as a test for the classification of the mass-forming cocci appears to show little promise. However, as suggested by the Winslows (1908), it may serve as a basis for the separation of the sarcina from the micrococci.

FERMENTATION OF SUGARS

Sugar fermentation has been used in classifying bacteria by many investigators and the results in some instances have been satisfactory. The action of the cocci on the various test sugars have been studied principally by Andrewes and Horder (1906) and by the Winslows

TABLE 11.—THE CORRELATION BETWEEN THE SUGAR FERMENTATION

FERMENTATION OF SUGARS	NUMBER OF CULTRES	HABITAT					GRAM				CHROMOGENESIS				DIASTATIC ACTION		LIQUEFACTION OF GELATIN		OXYGEN REQUIREMENT	
		Misc. parasite	Udder	Milk	Misc. saprophyte	Unknown	Positive	Var. gen'ly positive	Variable	Var. gen'ly negative	Negative	White	Yellow	Orange	Red	Positive	Negative	Positive	Negative	Facul'tive anaerobe	Microaerophilic
Dextrose																					
Acid	285	184	5	42	25	29	38	135	40	69	3	140	94	49	2	20	265	237	48	130	155
No acid	31	3	2	7	12	7	1	9	6	11	4	12	16	1	2	2	29	23	8	24	7
Lactose																					
Acid	258	174	4	40	21	19	29	125	36	65	3	126	82	48	2	18	240	219	39	109	149
No acid	58	13	3	9	16	17	10	19	10	15	4	26	28	2	2	4	56	41	17	45	13
Sucrose																					
Acid	254	173	4	30	25	22	24	121	33	50	6	136	69	47	2	14	240	209	45	102	152
No acid	62	14	3	19	12	14	15	23	13	30	1	16	41	3	2	8	54	51	11	52	10

(1908), both of whom noted the results quantitatively. The latter observers used dextrose and lactose only, while Andrewes and Horder used a number of sugars and other carbohydrates.

In the present work, the carbohydrates used were dextrose, lactose,

sucrose, glycerin, mannite, and ethyl-alcohol. In studying the relationships of this group to the various substances given above, it was found that lactose, dextrose, and sucrose gave the greatest amount of information. In the case of ethyl-alcohol no acid was produced by any of the cultures under investigation, whereas only a few produced acid from mannite, but in such cases the results were relatively constant. Similar results were also noted by Andrewes and Horder (1906). The results secured from glycerin, altho variable in some cases, indicate that this alcohol may be useful in the classification of this group of cocci. Dumas (1914), however, found only a small number of his organisms to attack glycerin.

In any system of classification or survey of a group of organisms the production of acids from test sugars should be interpreted as indicating the relative activities of the various organisms. The separation of groups on the basis of sugar fermentation in many cases, and especially in the case of the mass-forming cocci, is very undesirable as the results are not sufficiently constant and do not show definite correlations with any other character. The variations in this test are due in a large part to the variable conditions under which the test is made. For example, certain cocci among the strains being studied produced

AND OTHER CHARACTERS OF THE MICROCOCCI.

Reduction of nitrates		Amino nitrogen assimilation		Ammonia nitrogen assimilation		Ammonia production from				Hemolysis of blood		Maximum H-ion concentration dextrose broth				Action on milk						Nature of cells	
						Peptone		Nitrate								Acid		Rennet		Peptonization			
Reduced	Not reduced	Growth	No growth	Growth	No growth	Positive	Negative	Positive	Negative	No growth	Positive	Negative	pH 5.0 to pH 4.0	pH 7.0 to pH 5.0	pH 8.0 to pH 7.0	No growth	Sufficient to curdle	Not sufficient to curdle	No acid	Positive	Negative	Positive	Negative	Regular	Irregular
258	27	116	169	89	196	273	12	23	210	52	35	250	230	22	20	13	197	35	53	20	265	101	184	192	93
14	17	8	23	21	10	30	1	1	26	4	5	26	6	3	18	4	5	3	23	2	29	4	27	9	22
237	21	93	165	77	181	247	11	13	200	45	32	226	208	20	18	12	192	35	31	12	246	90	168	178	80
35	23	31	27	33	25	56	2	11	36	11	8	50	28	5	20	5	10	3	45	10	48	15	33	23	35
231	23	80	174	65	189	243	11	14	189	51	33	221	206	23	17	8	174	31	49	13	241	82	172	162	92
41	21	44	18	45	17	60	2	10	47	5	7	55	30	2	21	9	28	7	27	9	53	23	39	39	23

large amounts of acid from dextrose when inoculated from a 12-hour agar slant, but the same culture transferred from a 36-hour slant would produce no acid or would be very slow in acid production. The conditions of temperature, varying reactions of the media on

which the organisms are grown, and other factors also produce an important part in affecting the variation of the results.

The variation in the results was much greater in those cases in which peptone nutrient media were used than was true in cases where fermentation was observed on a synthetic medium containing no peptone or beef extract but with ammonium phosphate as the sole source of nitrogen. This fact is probably due, according to Ayers, Rupp, and Johnson (1919), to the formation of neutralizing substances from the peptone which are not produced from the ammonium phosphate.

It may be concluded, therefore, that sugar fermentation is not a satisfactory diagnostic character for use in separating natural groups of the mass-forming cocci but may serve as an indication of the relative activity of the various types.

GROWTH IN BROTH

In studying the characteristic growth of this group of cocci in liquid media, it was noted that two distinct types were found. First, organisms which gave a slight cloudiness to the broth with no precipitate; and second, a larger group which produced in addition to the cloudiness of the medium a heavy, persistent, and flocculent precipitate. The production of this precipitate by the free-living forms may be due to many factors as reaction of media, amount of inoculum, and various conditions of growth. The possible effect of these secondary factors on growth make it undesirable to select such a test for use in dividing groups into genera.

DIASTATIC ACTION

Various observers have found the action of different micro-organisms on carbohydrates very useful in grouping various strains of bacteria. Such classification, however, has been based primarily upon the production of acid from the sugars and various alcohols. In studying the action of bacteria upon starch, it would appear that starch, being a less available carbohydrate, would furnish a more resistant medium for the action of micro-organisms. The attacking of starch in such case would indicate the production of a diastatic enzyme.

In testing the action of the 316 cultures of cocci under observation upon starch, it was found that comparatively few, 22 in all, had the power to produce diastatic action. A repetition of the tests indicates

that the action on starch is very constant. The ability to attack starch, however, appears to show no relationship to any of the other characters studied and for this reason it is only applicable for use in differentiating smaller groups. Because of the small number of cultures which have this power it is questionable whether it can be used for the micrococci. Fermi (1890) decided that *Staphylococcus cereus flavus* produced a diastatic enzyme which differentiated this particular species from the rest of the group.

THE NATURE OF THE GROWTH ON AGAR SLANTS

Probably the most common and simple method of carrying cultures of various organisms is by means of agar slants. In earlier investigations and in various surveys and studies of certain groups of organisms, the nature of the agar slant growth has been used in definitely characterizing certain groups. In studying the nature of the growth of bacteria on agar slants, it has been generally thought that organisms may always produce the same type of growth under the same conditions of temperature, etc., and upon such conclusions certain groups have been separated and the characteristic beaded, filamentous, and various other types of growth are described as constant and characteristic of the strains thus separated. In the present work this has not been found to be the case, as it has been quite common for certain of the cultures thruout the course of this investigation to grow with a characteristic beaded growth under certain conditions, while under different conditions they produced an entirely different growth.

It was evident that both the growth on agar slants and colonies on agar plates showed no macroscopic features common to the group. Certain strains of this group under certain conditions have produced types of growth both on agar slants and plates very characteristic of the spore-formers, while other cultures have produced a growth on agar slants hardly distinguishable from streptococci and the more closely related organisms. Generally, however, the greater majority of the cultures under the same conditions will produce the typical "mass-forming cocci growth" on solid media. The amount of growth on agar slants appears to be more constant and much more significant. Cultures producing only scanty growth on the surface of ordinary nutrient agar under optimum conditions of temperature, etc., are very apt to produce the scanty growth under all conditions that permit growth, while those which produce abundant growth do so under all conditions.

The cultures producing an abundant growth on nutrient media were usually the saprophytic types, while the more scanty growers were largely parasitic. If the conditions affecting the growth of the cocci could be so regulated as to remain constant, the amount of growth as produced on nutrient media might serve as a satisfactory diagnostic character. However, these secondary growth characters are not sufficiently constant to warrant the use of the amount of growth as an important basis for classification.

OXYGEN REQUIREMENTS

Mass-forming cocci may be divided into two distinct groups according to their oxygen requirements, *viz.*, (a) those preferring aerobic conditions, and (b) those producing the best growth at the base of the agar stabs (microaerophilic). The first group or those which prefer oxygen includes most of the free-living types,

TABLE 12.—CORRELATION BETWEEN HEMOLYSIS OF BLOOD AND

	Number of cultures	Habitat					Gram					Chromogenesis				Diastatic action		Oxygen requirements		Liquefaction of gelatin	
		Misc. parasite	Udder	Milk	Misc. saprophyte	Unknown	Positive	Var. gen'ly positive	Variable	Var. gen'ly negative	Negative	White	Yellow	Orange	Red	Positive	Negative	Facul'tive Anaerobe	Microaerophilic	Positive	Negative
Action on Blood																					
Hemolyzed	40	32	0	2	4	2	4	18	6	12	0	29	8	3	0	0	40	19	21	37	3
No hemolysis	276	155	7	47	33	34	35	126	40	68	7	123	102	47	4	22	254	135	141	223	53
Action on Starch																					
Attack starch	22	9	0	4	5	4	3	8	8	3	0	5	13	3	1	—	—	15	7	20	2
Do not attack starch	294	178	7	45	32	32	36	136	38	77	7	147	97	47	3	—	—	139	155	240	54

while the second group is largely composed of the parasites. This relationship is variable, however, and many discrepancies can be noted in Table 4. No other apparent correlations could be found.

Lubinski (1894) states that parasitic staphylococci prefer anaerobic conditions, while Veillon (1893) and Hall and Frazer (1921) describe obligate anerobic cocci. The latter from the description, however, may be a streptococcus.

The mass-forming cocci appear to form a group midway between the obligate aerobes and the anaerobes, and being such a transition group in respect to oxygen requirements, it is not unlikely that such a character will be found suitable for classification.

HEMOLYSIS OF BLOOD

The use of blood hemolysis as a diagnostic test has been applied more largely to the streptococci than to any other of the closely related groups.

Kraus (1900), Neisser and Wechsburg (1901), Kraus (1902), Kolle and Otto (1902), Pröscher (1903), Klopstock and Bockenheimer (1904), Van Durme (1903), Veiele (1904), Kutscher and Konrich (1904), Lohr (1905), Fraenkel and Baumann (1905), Axenfeld (1907), Koch, J. (1908), Trincas (1908), Altmann and Schultz (1909), Jung (1909), Flügge (1910), Bruck and Hidaka (1911), Geisse (1913), Dumas (1914), Lyall (1914), Stevens and Koser (1919), Julianelle (1922), and others have demonstrated a hemolytic action among mass-forming cocci, and in most cases have done this with an idea of separating the group into two sub-groups using

DIASTATIC ACTION WITH OTHER CHARACTERS OF THE MICROCOCCI.

SUGAR FERMENTATION						REDUCTION OF NITRATES		AMINO NITROGEN ASSIMILATION		AMMONIA NITROGEN ASSIMILATION		AMMONIA PRODUCTION FROM			MAXIMUM H-ION CONCENTRATION IN DEXTROSE BROTH			ACTION ON MILK						NATURE OF CELLS		HEMOLYSIS OF BLOOD	
Dextrose		Lactose		Sucrose								Peptone		Nitrate				Acid		Rennet		Peptonization					
Acid	No acid	Acid	No acid	Acid	No acid	Reduced	Not reduced	Growth	No growth	Growth	No growth	Positive	Negative	Positive	Negative	No growth	pH 5.0 to pH 4.0	pH 7.0 to pH 5.0	pH 8.0 to pH 7.0	No growth	Sufficient to curdle	Not sufficient to curdle	No acid	Positive	Negative	Positive	Negative	Regular	Irregular	Positive	Negative
35	5	32	8	33	7	34	6	9	31	6	34	40	0	1	38	1	33	3	4	0	31	1	8	3	37	13	27	25	15	—	—
250	26	226	50	221	55	238	38	115	161	104	172	263	13	23	198	55	203	22	34	17	171	37	68	19	257	92	184	176	100	—	—
20	2	18	4	14	8	20	2	13	9	13	9	21	1	2	16	4	14	2	4	2	13	1	8	3	19	8	14	14	8	0	2
265	29	240	54	240	54	252	42	111	183	97	197	282	12	22	220	52	222	23	34	15	189	37	68	19	275	97	197	187	107	40	25

hemolysis as a basis for the separation. Orcutt and Howe (1922) have found that, altho the mass-forming cocci have, in some instances, a definite hemolyzing power, it varies in direct relation to the presence or absence of lipolytic enzymes. The addition of certain amounts of milk fats to the medium caused the hemolyzing power to vary and for this reason it is a question whether blood hemolysis can be used as extensively in classifying the mass-forming cocci as it has been in the case of the streptococci.

Of the 316 cultures studied only 40 hemolyzed blood (Table 12). In these instances, however, the hemolysis was clear cut and all of the *beta* type. Of the 276 which were found not to produce a hemolysis,

several were found which would produce a green area around the colonies very similar to that produced by *Streptococcus viridans*. The hemolyzing mass-forming cocci are naturally parasitic to a large extent and, being parasitic, are more or less Gram positive. Of the 40 strains which hemolyzed blood, 29 were white and a great majority of these white types were of the transparent variety, while none of the red types produced hemolysis. The hemolyzing cocci appear to be very active and especially is this true in relation to the liquefaction of gelatin and the production of acid in milk, while a large number produced a high maximum H-ion concentration in dextrose broth, and reduced nitrates but were variable in their relation to oxygen requirements.

There is no doubt a definite group of hemolyzing mass-forming cocci, which may be composed of one or more species, whose hemolytic activity is dependent upon some external factor.

NATURE OF CELLS

The mass-forming cocci when isolated from various pathologic lesions show on microscopic examination a characteristic regularity in size and shape of the cells. In such preparations the individual cells appear even in size and of practically the same diameter (0.5 to 1.0 μ) whereas in similar isolations the cocci from soil and other distinctly saprophytic sources show, in many instances, a different appearance. Preparations of organisms isolated from a saprophytic environment show many cells which are uneven in size and very irregular in shape, and the individual units of the same culture may vary from 0.5 to over 1.0 μ in diameter with many of the larger cells having the appearance of ovoid bodies. These and other observations might lead one to believe that the saprophytic cocci follow a more or less definite life history. Kocher and Tavel (1895) made several careful measurements of the mass-forming cocci while Lingelsheim (1900) and Rauolt-Deslongchamps (1897) noted also the uneven and irregular cells and suggested that reaction and composition of the medium may have an effect on the morphology of the cell. Matzuschita (1900) believed that the presence of certain salts in the medium may affect the size of the cells.

The probability that there is a life history in this group is also suggested when it is noted that the number of large bodies in the saprophytic cultures tend to vary at various stages in the growth of the culture. In the case of parasitic types, however, the character-

istic regular and even appearance of the cells is always found in all stages of growth. In order to determine, if possible, if there was any regular cycle of development among the micrococci preparations were made of each culture on the 1st, 2nd, 3rd, 5th, 7th, and 30th days of growth. Examination of these preparations indicates that certain groups of the mass-forming cocci pass thru more or less irregular stages which may be absent in closely related groups, as it is evident that certain changes in the morphology occur at the various intervals of growth. These changes, however, may or may not occur at regular intervals and future investigation will show their relationships and the possibility of a definite life history. From the above observations these changes are more noticeable among the saprophytic cocci than among the parasitic types, and it is possible that the saprophytes may pass thru a life cycle or they may be more sensitive to changes in external conditions which may affect their morphology. The parasitic cocci, on the other hand, are more constant; and if any definite morphological growth stages were present, they were not clearly demonstrated in any preparations examined in the present work.

Of the cultures, 115 (Table 9) were found to be more or less irregular in shape and 50 of these were from parasitic sources. A large majority of the 201 recorded as regular in shape were parasites. However, this character is not one that can be used as a basal character for the classification of this group.

SUPPLEMENTARY TESTS WHICH ARE LESS USEFUL IN THE CLASSIFICATION OF MASS-FORMING COCCI

Various observers who have studied the classification of cocci have used other tests than those discussed. In the preliminary series of tests mentioned above, the following characters were not found to be of importance in working out the systematic relationships of cocci. The results as found in the preliminary tests are given below.

FLAGELLA

Different observers have demonstrated distinct flagella on the cocci. Among these were Meyer (1903), Ellis (1902), Beijerinck (1901), Ali-Cohen (1889), Catterina (1903), Mendoza (1889), and others. Several strains of the cultures used in this survey were carefully stained at various intervals during their growth for the

presence of flagella and in no case were flagella demonstrated. However, it is possible that many cocci, and especially the free-living forms, possess flagella at certain stages in their growth under certain conditions.

MOTILITY

In connection with the demonstration of flagella the observers mentioned above have described cocci with a distinct and definite motility, while Glaser and Chapman (1912) have found a new motile coccus with sufficient constancy of motility to recommend a new genus, related to the Planococcus of Migula (1900). In studying some representative cultures for motility, negative results were found. Certain cultures, however, showed a movement which tended to be more or less of a rolling over and over across the field, but not the type of movement usually considered as characteristic of organisms which are definitely motile. The organisms, when examined at an early stage in growth usually showed this rolling motion and in all cases only a few of the organisms in each field presented this particular movement. It is possible that if the proper conditions could be established a larger number of the cocci would be found to be motile.

SPORE FORMATION

Beijerinck (1901), Ellis (1902), and others in addition to demonstrating flagella on certain strains of cocci have produced photomicrographs to substantiate their claims that the various cocci can, under certain conditions, produce definite spores. Babes (1895) has demonstrated the resistance of mass-forming cocci to drying on linen and silk threads, while Kirstein (1902) has demonstrated spore-like granules similar to those of Beijerinck. It is evident from a preliminary survey of the cultures under observation that certain granules are produced under various conditions in many cocci which have the general appearance of spores. Many of the strains showed these granules when a 7-day culture was stained with methylene-blue, and the resistance of these particular strains to heating and drying indicated that resistant bodies must be produced. In all probability these granules may be similar to the spores produced by other groups of bacteria. Even if definite spores are not produced, it is possible that the entire cell in certain strains of cocci may develop a heavy wall and become a spore-like body or arthrospore which is in-

distinguishable from the other cells when stained by our present staining methods.

PRODUCTION OF INDOL

A majority of the observers who have worked with this group of cocci have concluded that no indol is produced. Buard (1908), Selter (1909), Dobrowski (1910), Distaso (1911), Zipfel (1913), Herzfeld and Klinger (1915), Bayne-Jones and Zinninger (1921), and others have failed to find indol produced by the mass-forming cocci, while Tissier and Martelly (1902) found and reported an indol-producing coccus from meat. Emmerling (1896) described a similar organism which gave a positive indol test when grown on egg white for 14 days under anaerobic conditions. Distaso in 1912 isolated *Staphylococcus asaccharolyticus* (from the description this appears to be *Streptococcus fecalis*) which produced indol when grown in the absence of oxygen. In the present work the entire 316 cultures were tested for indol on trypsinized peptone broth by four methods (see above), and in no case was indol discovered.

THERMAL DEATH POINT

The power of the mass-forming cocci to resist heat has been noted in many instances. Russell and Hastings (1902) and their co-workers described in detail several micrococci which resist pasteurization temperatures in milk and in many cases a much greater amount of heat. Sternberg (1887) noted that staphylococci could withstand 62° C. for 10 minutes and 60° C. for 30 minutes, while Patzchke (1916) found cocci resisting 80° C. for 1 to 2 seconds, 65° C. for 30 to 60 seconds, 60° C. for 5 to 10 minutes, and 50° C. for 1 hour and longer. In the series of cultures collected for this study, as stated above, several were isolated from pasteurized milk and the ability of these cultures to resist heat is very striking. Certain of the cultures possessed the power to resist 145° F. (62-3° C.) for 30 minutes, and some cultures even greater temperatures. This particular characteristic of this group of cocci has a decided economic bearing. In pasteurizing plants where 140° to 145° F. for 20 minutes is used to reduce the bacterial count in milk, it has been noted in several instances that large numbers of these heat-resisting micrococci are present in milk after such pasteurization. The presence of this heat-resisting power in certain strains of these cocci furnishes a further indication that spores or spore-like bodies may be produced.

The resistance of the micrococci to heat is no doubt a very variable character similar to the effect of various disinfectants upon members of this group, and by further study certain contributing factors may be found which play an important part in the general resistance of the micrococci to heat, drying, chemicals, etc. Lübbert (1886) and Paul and Prall (1907) have found that micrococci could resist temperatures as low as that of liquid air.

AGAR COLONIES

The types of colonies produced by cocci are very characteristic, but a few colonies are so variant in type that this character cannot be adopted in definitely separating cocci from other groups. Serkowski (1901) and Saul (1900) have given detailed descriptions of colony types, but it seems improbable that such minute differences can be relied upon as constant, while Catterina (1903) described a micrococcus producing a moss-like colony.

In general, however, the more free-living types, due to their

TABLE 13.—A COMPARISON BETWEEN THE

	Number of cultures	Habitat					Gram					Chromogenesis				Liquefaction of gelatin		Diastatic action		Oxygen requirement	
		Misc. parasite	Udder	Milk	Misc. saprophyte	Unknown	Positive	Var.gen'ly positive	Variable	Var.gen'ly negative	Negative	White	Yellow	Orange	Red	Positive	Negative	Positive	Negative	Facul'tive anaerobe	Microaerophilic
Cell grouping																					
Micrococci	316	187	7	49	37	36	39	144	46	80	7	152	110	50	4	260	56	22	294	154	162
Sarcinae	20	0	0	3	5	12	2	7	3	8	0	0	18	0	2	19	1	2	18	20	0

aerobic tendency, grow in circular colonies with a characteristic moist appearance; while, on the other hand, the more anaerobic parasitic types produce deep punctiform colonies. Certain Gram negative, pigmented rods and other types produce colonies very similar to those produced by this group, thus making it impossible to differentiate accurately the micrococci from closely associated organisms.

SLIMINESS

Various strains of this group produce a slimy growth on agar streaks and many times in milk and other liquid cultures. Certain

investigators have used this character as the basis for the formation of new species. This characteristic, however, seems to vary, and in some cases old cultures, particularly of *M. albus*, of non-slime-producing strains, will produce a slimy and ropy growth which can be drawn from the growth in long threads. Some unknown factor seems to control its production, and until more is known as to its nature the development of sliminess should not be used as a basis for the formation of new species in the group of micrococci.

COMPARISON OF THE CHARACTERS OF THE SARCINAE AND MICROCOCCI

The grouping of the cells of the cocci have furnished for some time a character upon which large groups of cocci have been separated. Since Goodsir (1842) recognized the packet forms and Billroth (1874), and others the chain types, the arrangement of cells into more or less irregular packets has been used to characterize the mass-forming

CHARACTERS OF SARCINAE AND MICROCOCCI.

SUGAR FERMENTATION			REDUCTION OF NITRATES	AMINO NITROGEN ASSIMILATION	AMMONIA NITROGEN ASSIMILATION	AMMONIA PRODUCTION FROM		HEMOLYSIS OF BLOOD	MAXIMUM H-ION CONCENTRATION IN DEXTROSE BROTH	ACTION ON MILK			NATURE OF CELLS
Dextrose	Lactose	Sucrose				Peptone	Nitrate			Acid	Rennet	Peptonization	
Acid	Acid	Acid	Reduced	Growth	Growth	Positive	Positive	Positive	pH 5.0 to pH 4.0	Sufficient to curdle	No acid	Positive	Regular
No acid	No acid	No acid	Not reduced	No growth	No growth	Negative	Negative	Negative	pH 7.0 to pH 5.0	Not sufficient to curdle	Positive	Negative	Irregular
								No growth	pH 8.0 to pH 7.0	No acid	Negative	Positive	
									No growth				
235	258	254	272	124	110	206	303	13	24	236	56	40	276	236	25	38	17	202	38	76	22	294	105	211	201	11
8	10	8	12	20	18	2	17	3	4	14	2	3	17	5	2	10	3	4	2	14	9	11	13	7	3	
31	58	62	44	192	206	303	13	24	236	56	40	276	236	25	38	17	202	38	76	22	294	105	211	201	11	
12	10	12	8	0	2	17	3	4	14	2	3	17	5	2	10	3	4	2	14	9	11	13	7	3		

cocci. The production of tetrads does not appear to be as constant a character as chain or packet formation and special groups or species separated upon this character alone have not been considered as important from a taxonomic standpoint.

The micrococci are separated from the streptococci by other characters in addition to that of differences in the methods of cell division, such as type of growth on agar, habitat, food requirements, chromogenesis in part, etc. The sarcinae, however, appear to be closely related to this group, and in order to secure additional data on the relationship of these two groups of cocci, the 316 cultures of

micrococci which were studied were compared with 20 representative strains of sarcinae obtained from various laboratories.

Except for the differences in the arrangement of cells the differentiation of the sarcinae and micrococci has been difficult as the type of growth, chromogenesis, sugar fermentation, etc., are very similar in many cases. The formation of definite packets, however, has been considered by nearly all systematists as a sufficient reason for separating the sarcinae, while the Winslows (1908) suggest that the packet formers may also be more rapid ammonifiers than the micrococci.

Table 13 shows a comparison of the general characters of these two groups. It is interesting to note that there appears to be no definite and clear cut line of division other than that of packet formation.

The sarcinae tend to be a little less active in milk, are generally faculative anaerobes, and are usually saprophytes. However, the formation of definite packets appears to be a constant feature of the sarcinae which is not present in the micrococci. Many workers have suggested that the latter group would form packets under proper conditions, *viz.*, in liquid media. The entire group of cultures of this series was examined microscopically in broth at various ages and in no instances were constant and definite packets found.

In examining this group in a liquid medium, however, it was noted that most of the cultures produced definite tetrads, while the same cultures examined from solid media gave only an occasional indication of tetrads. The above observation is interesting in the light of the fact that microscopic preparations made of market milk samples and other liquids frequently show large numbers of tetrads, but upon plating these samples and isolating the various coccus cultures it is usually impossible to find tetrad-forming strains when subsequently grown on agar slants.

In one instance (*Micrococcus tetragenus*) an exception is found as this particular culture forms definite tetrads under practically all conditions.

From this it appears that the production of definite packets is a character sufficiently constant and important to be useful in separating the sarcinae from the micrococci but the two genera are without question very closely related.

THE EVOLUTIONARY DEVELOPMENT OF THE MICROCOCCUS GROUP

A study of the systematic relationships of any group of plants or animals involves grouping individual organisms with a common origin into what are known as "natural species." Groups of closely related species are then, for convenience sake, associated into larger groups called genera, the species however remaining as the basic unit. In order to apply this principle to this group of cocci, we must at least speculate as to their geological origin and associate species which appear to have a similar developmental origin together into genera.

Biologists generally agree that the most primitive habitat was either the ocean or soil as primitive organisms originated in salt water or soil. Certain very apparent difficulties are met when an attempt is made to select the most primitive form of all bacteria, as several groups may have originated from one hypothetical primitive form. Of the micrococcus group, however, no doubt the white, non-liquefying, and non-nitrate-reducing types which have the ability to utilize inorganic sources of nitrogen are to be regarded as the most primitive. This original coccus may or may not have been motile, but later probably developed motility. As the habitat became more specialized in or on solid substances, however, motility became non-essential and the power of locomotion was lost.

In the earlier forms of life the power of assimilating organic nitrogen was no doubt lacking as ammonium salts and other inorganic compounds were the only sources of this element. Later, however, upon the advent of the more specialized animals and plants organic sources of food became more abundant and these primitive bacteria, being very plastic in their requirements, soon developed the ability to utilize such forms of food which proved to be much more economical than their previous inorganic sources. The more highly specialized forms gradually lost this power of assimilating inorganic nitrogen and became adapted only to living in the presence of organic materials.

The evolutionary development of the pigmented forms presents some difficulties for, on the one hand, the pigment may have been developed as an aid in synthesizing certain compounds in the presence of light rays or, on the other hand, the pigment may be an excretory product. It is barely possible that with the changing characteristics of the organism certain foods were added to the changing require-

ments of the organisms that gave as a product in their metabolism these colored substances so commonly associated with this group of cocci. The red and yellow cocci which are invariably found in saprophytic sources may find some particular substance which has become at least an important part of their diet, if not an essential constituent, which contributes to the characteristic color of this group, while the more highly specialized species which have become more or less parasitic in habitat have a similiar unknown food which contributes to the orange color developed in this case.

It is no doubt probable that this group of micrococi originated as free-living, saprophytic forms and acquired the specialized characteristics of parasitism as in those types now found commonly associated with mammalian bodies. The utilization of ammonium salts by the more saprophytic forms indicates that certain types still retain the power to secure nitrogen from such sources. The change from this primitive form to the more highly developed types has been so gradual and so many transition forms are still present that it is impossible to separate the species, represented in the group, into genera.

CONCLUSIONS

From the foregoing data, it appears that the irregular, mass-forming cocci, generally known as the staphylococci or micrococci, form a single group which does not appear to be subdivided into two or more sub-groups of generic rank. The division of this group on the basis of habitat or chromogenesis, as followed by many of our present day systematists, gives genera which do not have definite border lines, as cultures that are apparently identical in other respects are placed in separate genera. In the studies that have been made, no correlations of characters were found that indicated that there were natural subdivisions of this group.

Several genera of the irregular mass-forming cocci have been formed in the past on the basis of chromogenesis (*Albococcus*, *Aurococcus*, *Micrococcus*, as emended by the Winslows, *Rhodococcus*, etc.), but in practically all instances such large numbers of exceptions have been found that these genera have been abandoned, and in the latest classifications only the red micrococci have been retained in a separate genus. It seems inconsistent, however, to separate this particular group on such a basis when all other chromogenic micrococci are retained in a single genus or are grouped with little regard to chromo-

genesis. A study of the cultures included in this survey and also of many other strains secured subsequently fails to show any characteristic which is correlated with the production of red pigment. Apparently, the known facts do not warrant separating these cocci from other closely related cocci.

The division of the irregular, mass-forming cocci on the basis of habitat, as is frequently done, meets with equal difficulty. The resulting groups are not natural ones. The habitat of organisms is a poor characteristic to use as a fundamental one in separating genera and such a classification rarely produces natural groups with the same developmental origin.

The irregular, mass-forming cocci form a more or less homogeneous group in which the individual cocci ordinarily divide in two or three planes. The resulting units, however, do not remain in position so that an irregular mass formation results. Chains of cells are rarely formed and then only under certain conditions. These cocci are chiefly to be found on the outer or inner surfaces of mammalian bodies or in the skin glands, including the mammary glands, also in lesions. They also live commonly in milk and in dairy products and utensils. They are found in air and water, and less commonly in soil and plant tissues. Pigment may or may not be produced and when present is either red, orange, or yellow. The group as a whole does not secure its energy from a wide range of carbohydrates and is not sensitive to minor variations in its food supply. Abundant growth is produced on all ordinary laboratory media.

Chromogenesis, nitrate reduction tests, gelatin liquefaction, action on milk, and ability to utilize ammonium phosphate as a sole source of nitrogen, altho not tests of generic value, are of primary importance in separating the group into sub-groups and species.

Reaction to the Gram stain, fermentation of carbohydrates, habitat, hemolysis of blood, ammonia production, etc., are less constant and, therefore, are of less value for classification purposes. In certain cases, however, these characteristics are very useful in distinguishing individual species.

As the present investigation does not indicate more than 16 distinct species among the cultures studied, the retention of all of the irregular, mass-forming cocci in a single genus *Micrococcus* does not produce a large or unwieldly genus.

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